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A Passive Acoustic and Experimental Study of Juvenile Blue Catfish, Ictalurus furcatus, Sound Production and Agnostic Behavior in the Tidal Freshwater James River

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A PASSIVE ACOUSTIC AND EXPERIMENTAL STUDY OF JUVENILE BLUE CATFISH, *ICTALURUS FURCATUS*, SOUND PRODUCTION AND AGONISTIC BEHAVIOR IN THE TIDAL FRESHWATER JAMES RIVER

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.
by

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Genera Abstract

A PASSIVE AND EXPERIMENTAL STUDY OF JUVENILE BLUE CATFISH, ICTALURUS FURCATUS, IN THE TIDAL FRESHWATER JAMES RIVER

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Blue catfish, *Ictalurus furcatus*, are an invasive species in the James River, VA. They produce stridulation sounds and passive acoustic monitoring may prove useful in locating and monitoring their populations. Little is known about their behavior, therefore my goal was to examine agonistic behavior and the use of sound in defending a territory. This thesis consists of two manuscripts: 1) A passive acoustic study of the tidal freshwater James River, 2) An experimental study of agonistic behavior in juvenile Blue catfish, *Ictalurus furcatus*. The first study showed that three sounds (click, run croak) occurred more often in warmer months than cooler months. The second study showed that Blue catfish utilized a variety of agonistic behaviors in territory defense, with residency status and size having an effect on the type and number of displays used. Stridulation sounds were not present in territorial contests although Blue catfish produce stridulatory pulses when held.

General Introduction

This thesis contains two chapters. The first is a passive acoustic study of the tidal freshwater James River and the use of passive acoustic monitoring (PAM) in monitoring populations and periodicity of organisms. The second chapter is an experimental study of agonistic behavior in juvenile Blue catfish.

The Blue catfish as a species of concern

The Blue catfish, *Ictalurus furcatus*, an invasive species within the James River and other Chesapeake Bay tributaries, is currently found in 29 states due to migration and introduction (Graham and DeiSanti 1999). Blue catfish were introduced into the James River from the Mississipi River in 1974 to provide recreational fishing opportunities. They currently pose a health concern to residents who consume their meat, as their tissues exhibit high concentrations of polychlorinated biphenyls and tributyl tin (Harris and Jones 2008; Weintraub 2008). This leads to apprehensions over their current protection for sport fishing and the negative impact they have on the ecosystem.

Blue catfish populations have boomed causing a negative impact on the local wildlife. They are successful in part because they can move large distances (Lagler 1961) and withstand up to 11 % salinity for short periods (Perry 1968). In the James River they have an annual mortality rate of 26.5 %, lower than the average from other rivers, and they have increased in density with little negative effect on their growth rate compared to nearby rivers (Greenlee and Lim 2011). The species now comprises approximately 75 % of the fish biomass in the tidal freshwater James River (Schloesser et al. 2011).

Blue catfish numbers correlate with decreasing native white Catfish populations (Schloesser et al. 2011). Furthermore, Blue catfish may be contributing to declines in shad spawning migrations (MacAvoy et al. 2000) despite intense stocking efforts. Therefore a better understanding of the Blue catfish will aid in management techniques that may help both native wildlife and Virginia residents.

Blue catfish are a valued recreational fish and breeding stock for Channel catfish hybridization. At least half of the states outside of the natural range of the Blue catfish value them as recreationally important (Graham, 1999). In the United States catfish are the highest grossing aquaculture fish (USDA, 2005), making them a prized monetary source. Channel catfish, *Ictalurus punctatus*, are often farmed; however Blue catfish are unpopular in aquaculture because of slow maturation, poor conversion of food, and low spawning rates in captivity (Graham, 1999). We have observed that these fish can become highly aggressive and can inflict spine damage when housed together. When Blue catfish are hybridized with Channel catfish, they produce offspring with greater dress out and fillet percentages (Argue et al. 2003). Because Blue catfish do not readily hybridize with Channel catfish, artificial spawning must be used (Masser and Dunham 1998). Understanding their behavior could be important in the farming of this and other species of catfish.

The ecology of Blue catfish has been well studied. Blue and Channel catfish consume a variety of foods including mussels, clams, fishes, and invertebrates (Perry 1969; Schloesser et al. 2011). Fish consumption starts when Blue catfish reach 100 mm in total length, and they become exclusively piscivorous at 290 mm (Perry, 1969). Blue catfish consume large numbers of anadromous fishes, which migrate into freshwater for reproduction (MacAvoy et al. 2000) causing them to be classified as an invasive species. Blue catfish are bottom dwellers that prefer deep, cloudy, and fast flowing water with a gravel-sand, silt-mud substrate (Burr and Warren 1986).

The reproductive habits of Blue catfish are not well understood, and their size and age at their initial spawning season vary geographically (Perry and Carver 1973; Graham and DeiSanti 1999). Blue catfish have a higher hatching success and fry production than Channel catfish (Tave and Smitherman 1982). They are cavity nesters (Pfleiger 1997), and the male guards the eggs and small fry. Blue catfish mature at 4-5 years of age, or about 381 mm total length, in the mid-Atlantic (Barnickol and Starett 1951) and earlier in the southern parts of their range (Henderson 1972; Perry and Carver 1973) likely due to warmer weather and higher diversities of food sources.

Behavior and Sound Producing Abilities

With over 3,000 species, Catfishes include about one-third of all freshwater fishes making them among the most successful groups of fishes (Teugels, 2003). This success is likely due to highly developed chemical and auditory (Caprio and Finger 2003; Ladich and Bass 2003) rather than visual ability (Collin 2003). Motor specializations hinge primarily on adaptation of the pectoral spine that can be locked in place as an anti-predator adaptation (Fine and Ladich, 2003; Fine et al., 1997). Additionally, the dorsal process at the base of the pectoral spine can be rubbed against a channel in the pectoral girdle producing stridulation sounds (Fine et al., 1997; Kaatz et al., 2010). They produce pulses with frequencies between 1,000-8,000 Hz, with a majority of the energy concentrated around 1,000-4,000 Hz (Ladich and Myrberg, 2006). Many tropical Catfishes also produce sounds with extrinsic muscles that deform the swimbladder (Fine and Ladich, 2003; Kaatz et al., 2010), but the North American Ictaluridae do not produce swimbladder sounds (Fine et al., 2011b).

The Fine lab has worked extensively on the pectoral spine as an anti-predator adaptation (Bosher, Newton, and Fine 2006; Sismour et al., 2013), the effects of predators on growth, feeding, and movement of Channel catfish (Fine et al., 2011a), and on mechanisms of sound production (Fine et al., 1996; Fine et al., 1997; Fine et al., 2011b; Ghahramani, 2010). We have evoked sounds in channel and Blue catfish by holding them but have not investigated the incidence of sounds in nature or the function of these sounds. Since nearly 100% of Blue catfish will produce sounds when held (Ghahramani, 2010), it is likely that acoustic communication is important in this species.

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CHAPTER 1:

A PASSIVE ACOUSTIC STUDY OF THE TIDAL FRESHWATER JAMES RIVER

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Abstract

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Passive acoustic monitoring (PAM) has been used to document the daily and seasonal periodicity of marine and freshwater mammals, fishes, and invertebrates in their environments. Only a single PAM investigation on North American freshwater tidal rivers, the Hudson River, has been published. I recorded and analyzed seasonal and diel incidence of sounds in the tidal freshwater James River. Twenty-five different sound types were present: three occurred commonly and twenty-two occurred uncommonly. Three sounds (runs, clicks, and croaks) occurred hrly nearly every month although the greatest activity occurred in summer. The clicks are likely from Atlantic sturgeon, and the presence of year-round sounds suggest that these sounds were made by young fish since spawners migrate into the James in the spring and fall. Of the uncommon sounds, more types and numbers were found in April and May**.** Our freshwater system showed less sonic activity than marine and tropical environments.

Introduction

Aquatic environments host a wide variety of sound producing organism. Many aquatic insects (Sueur et al., 2011) and crustaceans, such as crayfish, lobster, and shrimp produce sounds (Favaro et al., 2011; Meyr-Rochow and Penrose, 1976; Johnson et al. 1948). Over 700 species of fish can produce swimbladder or stridulation sounds (Ladich, 1997; Kaatz, 2012). Mysticete (Adam et al. 2013; Green et al. 2011) and Odonticete (Cranford et al., 2011) whales can produce both communication and echolocation sounds.

Passive acoustic monitoring (PAM) has been used in a range of applications that allow an accurate picture of circadian rhythms to be taken. Experimental methods under captive settings, can alter activity patterns and behavior (Calisi and Bentley, 2009, Boujard and Leatherland, 1992) and are not well suited for behavior or activity studies. Remotely operated vehicles (ROV) allow for mobility and optic attachments to aid in identification of unknown sounds through photographs of nearby species. ROV’s can disturb fish and create background noise (Rountree and Juanes, 2010; Wall et al. 2012). Stationary platforms can detect silverperch and weakfish spawning grounds at distances of 1 km under ideal conditions (Luczkovich et al., 2008) and choruses of fish and invertebrates up to 2 km away (D’Spain and Batchelor, 2006). PAM can shed light on circadian rhythms (Wall et al., 2013). PAM has applications for the identification of species, use of habitat, and tracking movement of fishes and invertebrates (Di Iorio et al. 2012; Luczkovich et al. 2008).

Blue catfish, *Ictalurus furcatus*, are an invasive species within the James River, VA and other Chesapeake Bay tributaries. They compromise approximately 75% of the fish biomass within the tidal freshwater James River (Schloesser et al., 2011) and are associated with decreases in shad populations during spawning migrations (MacAvvoy et al., 2000) and native white Catfish populations (Schloesser et al., 2011). Because North American Catfishes in the family Ictaluridae produce stridulation sounds (Kaatz et al., 2010; Fine et al., 1997; Ghahramani, 2010) we used PAM to examine Blue catfish sonic activity. Although Channel catfish, *Ictalurus punctatus*, will produce disturbance sounds when held (Fine 1996; 1997), only one paper has been published on naturally-occurring sounds of North American catfish. This study on the brown bullhead indicates that sound plays a role in submissive behavior (Rigley and Muir, 1979).

Sounds of North American tidal freshwater systems have been neglected except for a single study that examined nocturnal sounds in the Hudson River during three months at the end of summer (Anderson et al. 2008). Investigations of marine environments reveal that they are relatively noisy (Wall et al., 2014; Wenz, 1961, Woillez et al., 2012) and assessments of freshwater systems in South America also indicate high sonic activity (Ding, Wursig, and Leatherwood, 2001; Borie et al., *submitted*).

The goal of this project was to categorize sounds and their daily and seasonal occurrences in the freshwater James River with particular emphasis on the contribution of Blue catfish to the freshwater sounds.

Materials and Methods

Passive Acoustics

Underwater recordings were made from February 2012-January 2013 (although December and January's data were lost due to a corrupted harddrive) in the tidal freshwater James River with a HTI (model 96-Min) (sensitivity of –165 dB re: 1V/uPa) connected to a Wildlife Acoustics Song Meter (model SM2) recorder. Recordings were sampled at 16,000/min with no amplification. I made 10 min recordings hrly for 48 hrs every month from the Rice Rivers Center Pier (Charles City, VA) 119 km from the Chesapeake Bay. Temperature data were taken every 15 minutes from a YSI 6600eds monitoring sond hung from the same dock.

Recorded sounds from the first 3 min of each hr were analyzed with Raven Pro v1.3 software. Examination of the full 10 min indicated few new sounds and similar incidences of sounds after 3 min. I therefore sampled for three min at the beginning of each hr. Selections with high background noise levels were replaced with another selection from the same hr. Oscillograms and spectrograms were obtained for each type of sound using Raven. Peak frequency and duration were measured on 20 samples of commonly occurring sounds. Power spectra employed a Hann window (sample size: click=940, run=1758, croak=1118, 16.5 Hz bandwith filter, DFT size=2048, and grid spacing=7.81 Hz) on select sounds. To increase sampling due to short time window, 10 clicks were assembled together and an FFT was run on this collective sample. Known abiotic sounds were not included for analysis; these included drilling on the pier, knocking of crab pots on ladder, and boat motors.

Statistics

A non-parametric 1way ANOVA (Kruskal-Wallis) with a Dunn's Multiple Comparison test was used to compare seasonal occurrences of the three most common sounds. A linear regression was used to compare mean occurrences versus temperature. We compared seasonal, but not diel, occurrences from four less common sounds. All other sounds occurred to infrequently to categorize seasonal or daily occurrences.

Results

Twenty-five different distinct sound types were recorded in the tidal freshwater James River. Three sounds (clicks, runs, and croaks) occurred every month and 22 sounds occurred sporadically.

Clicks had a peak frequency of 5356.3 ± 386.3 Hz (Mean ± SE) and lasted 13.4 ± 3.0 ms (Figure 1). Clicks were nearly indistinguishable from the background noise on the power spectrum (Figure 2). In 7 of 10 months, clicks occurred mostly during the day. In May and August activity peaked at dusk and dawn (Figure 37). Clicks activity divided into three statistical groups based on hrly occurrence per month (Kruskal Wallace Statistic (KW)=149.9, $p<0.0001$): A (February, March, April, November), B (March, April, July, October), and C (May, June, August, September). May had the most activity (86.3 ± 4.8) followed by August (65.7 ± 4.6). February had the least activity (5.5 ± 1.9) followed by November (18.6 ± 3.1) (Figure 32).

Runs had a peak frequency of 162.5 ± 16.4 Hz, and lasted 91.9 ± 6.6 ms (Figure 3). Amplitude peaked at 70, 345, and 420 Hz (Figure 4). In 7 of the 10 months runs occurred before dawn or dusk (7 months) (Figure 38). Runs activity was divided into four statistical groups based on hrly occurrence per month (KW=126.0, $p<0.0001$): A (February, March), B (March, April), C (April, May, October, November), and D (May-November). November had the most activity (122.8 ± 45.6) followed by June (54.9 ± 5.4), and September (54.1 ± 4.7). February had the least activity (0.3 ± 0.3) followed by March (7.9 ± 2.5) (Figure 32).

Croaks had a peak frequency of 332.8 ± 48.2 Hz and lasted 637.3 ± 69.4 ms. Croaks showed peaks at 270, 490, 720, and 1,610 Hz (Figure 6). Croaks were more common between 6 and 11 AM than between midnight and 5 AM (KW=15.72, p=0.0013) (Figure 33). Clicks activity divided into four statistical groups based on hrly occurrence per month (KW=109.9, p<0.0001): A (February, March, October, November), B (March, April), C (April, May, July-November), and D (June-September). June had the most activity (5.9 ± 0.8) followed by July (3.8 ± 0.5). February had the least activity (0.04 ± 0.04) followed by March (1.2 ± 0.4) (Figure 32).

The incidence of clicks (p=0.0141, r^2=0.5502) and croaks (p=0.0055, r^2=0.6388) increased in occurrence with increasing temperatures. There was an aberrantly high number of runs in November. The incidence of runs (p=0.0045, r^2=0.7065) also showed and increase with increasing temperatures (not counting November). Run and croak both show a curved fit with increasing occurrences into the late summer and decreasing occurrences into the fall (Figure 34).

For the 22 less commonly occurring sounds, diversity and number peaked in April and May (Figure 36). Of the less commonly occurring sounds, Sound 1, 3, 4, and 12 had enough occurrences to check for periodicity. There was no seasonal periodicity for Sound 1 difference (KW=10.44, p=0.3162). For Sound 3 there was an increase between February and March through August, and October (KW=57.4, p<0.001). For Sound 4 there was an increase in early summer and again through the fall (KW=24.18, p=0.0040).For Sound 12 there was an increase in activity in May and June, with decreasing activity through November with a peak in October (KW=54.28, p<0.0001) (Figure 35).

Discussion

Although it was previously believed otherwise; freshwater appears to have a quieter sonic landscape than marine environments (Miksis-Olds et al. 2013). Sounds within North American rivers have not been well studied, and only a single paper exists on this topic (Anderson 2008) leaving this a poorly explored area. Sixty two different sounds were found in this study, including many abiotic sounds and 25 distinct biotic sounds were detected in my study. There are 69 known ray finned fish species from 19 families in the tidal freshwater James River (Table 3) and it is believed that many of the lower frequency sounds come from these species.

This study is the first to examine seasonal and daily periodicity in 3 sounds, and seasonal shifts collectively in the other sounds. All sounds showed an increasing trend in sonic activity through the summer, although the peak months varied for individual sounds. Increasing temperature correlated with an increase in sound production with a slight decrease in the warmest temperatures (31.5^{O} C), possibly due to decreased activity (Fry, 1947). Higher temperatures have been shown to increase swimming and aggressiveness in some species of fish (Hess, 1952).

Species and sound variability shift with time and temperature. Species diversity of fish is often greatest in the summer months and lowest in winter months with intermediate numbers in fall and spring, which are considered to be transitional periods (Reina-Hervaas and Serrano 1987; Mukherjee et al. 2013). Sound occurrence and type can change seasonally for a species and this sonic shift is likely attributable to reproduction (Connaughton, and Taylor 1995). For example w=Weakfish, *Cynoscion regalis*, transitioned from drumming to chattering in its reproductive season of June and July (Morano et al. 2012).

Periodicity in sound production is poorly documented in a majority of fishes (Reebs, 2002) and varies across species. The longspine squirrelfish defends its territory with acoustical displays and activity and vocalization pattern peaks in the crepuscular period (Winn et al. 1964), similar to the organism producing the run sound. The hrs after dawn show and increase activity in many species of fish (Fanta, 1997; Schwassmann, 1971). Light may be responsible for this periodicity of activity and sound production (Boujard 1995; Kasai et al.2009).

Clicks are similar to ones recorded by Phillips et al. (unpublished) from the Gulf Sturgeon and possibly come from Atlantic sturgeon, *Acipenser oxyrinchus*. The absence of likely identifications of the other sounds indicates our ignorance of the acoustics of tidal freshwater systems. The absence of stridulation sounds from Blue catfish was surprising given their abundance in this area **(**Schloesser et al. 2011), suggesting that their sounds are not important in courtship, assuming the fish in the area were spawning. Similar sounds were not present in agonistic behavior of juveniles (Morgan 2014), and therefore sound production in Blue catfish may be restricted to distress calls produced during capture by a Largemouth Bass(Bosher et al. 2006; Ladich and Myrberg 2006; Matthis et al 1996)

Table 1: Sunrise, Sunset, and Mean Water Temperature for monthly recordings

Date (2012)	***Sunrise (AM)***	***Sunset (PM)***	***Water Temperature***
2/17	6:47	5:51	8.5
3/30	5:58	6:31	18
4/27	5:19	6:57	21
5/19	4:57	7:16	27.5
6/27	4:51	7:35	29.5
7/13	5:00	7:31	31.5
8/2	5:15	7:16	29.5
9/5	5:44	6:32	27.5
10/23	6:58	4:54	17.5
11/16	7:18	4:53	10.5

Table 2: Total Occurrences of less common sounds within 3 min over 24 hrs from February-November 2012 in the tidal freshwater James River

Sound Number	February	March	April	May	June	July	August	September	October	November
1	1	1	5	7	11	1	5	3	1	10
2	0	0	2	3	0	3	0	0	1	0
3	0	3	34	49	24	14	23	10	33	20
4	0	13	3	25	10	0	6	6	13	18
5	0	6	0	0	0	0	0	0	0	0
6	0	0	2	0	0	0	3	0	0	0
7	0	4	0	22	3	0	27	0	7	0
8	17	0	0	0	0	0	0	0	0	0
9	0	12	0	0	0	0	0	0	0	0
10	0	0	0	50	0	0	0	0	15	0
11	0	1	0	3	0	0	0	0	0	0
12	0	0	7	42	29	0	10	4	32	10
13	15	0	0	0	0	0	0	0	0	0
14	2	0	0	0	0	0	0	1	0	0
15	0	0	15	4	4	0	0	0	0	0
16	7	0	1	1	0	1	1	0	0	0
17	4	0	9	12	4	1	11	1	2	3
18	0	1	0	0	3	1	1	4	12	1
19	0	0	3	4	1	0	0	0	3	0
20	0	0	6	1	2	2	1	0	0	0
21	0	0	0	0	0	29	4	4	0	2
22	5	0	1	2	0	0	0	0	3	0

Table 3: List of known ray finned fish species within the tidal freshwater James River (Viverette 2004).

Acipenseridae	*Acipenser oxyrhynchus*	Atlantic Sturgeon
Lepisosteidae	*Lepisosteus osseus*	Longnose Gar
Amiidae	*Amia calva*	Bowfin
Anguillidae	*Anguilla rostrata*	American eel
Clupeidae	*Alosa aestivalis* *Alosa mediocris* *Alosa sapidissima* *Alosa psuedoharengus* *Brevoortia tyrannus* *Dorosama cepedianum* *Dorosama petenense*	Blueback herring Hickory shad American Shad Alewife Atlantic menhaden Gizzard shad Threadfin shad
Engraulidae	*Anchoa mitchilli*	Bay anchovy
Esocidae	*Esox niger*	Chain pickerel
Cyprinidae	*Cyprinella analostana* *Cyprinus carpio* *Hybognathus regius* *Nocomis raneyi* *Notemigonus crysoleucas* *Notropis amoenus* *Notropis hudsonius* *Notropis procne* *Semotilus corporalis*	Satinfin shiner Common carp Eastern Silvery minnow Bull chub Golden shiner Comely shiner Spottail shiner Swallowtail shiner Fallfish
Catostomidae	*Carpiodes cyprinus* *Catostomus commersonii* *Erimyzon oblongus* *Hypentelium nigricans* *Moxostoma erythrurum* *Moxostoma macrolepidotum*	Quillback White sucker Creek chubsucker Northern hogsucker Golden redhorse Shorthead redhorse
Ictaluridae	*Ameiurus natalis* *Ameiurus catus* *Ameiurus nebulosus* *Italurus furcatus* *Ictalurus punctatus* *Noturus insignis* *Plyodictis olivaris*	Yellow bullhead White catfish Brown bullhead Blue catfish Channel catfish Margined madtom Flathead catfish
Aphredoderidae	*Aphredoderus sayanus*	Pirate perch

Table 3: (continued).

Antherinidae	*Membras martinica* *Menidia beryllina* *Menidia menidia*	Rough silverside Inland silverside Atlantic silverside
Fundulidae	*Fundulus diaphanus* *Fundulus heteroclitus*	Banded Killifish Mummichog
Poecilidae	*Gambusia holbrooki*	Easter mosquitofish
Mugilidae	*Mugil cephalus*	Striped mullet
Moronidae	*Morone americana* *Moronose saxatilis*	White perch Striped bass
Centrarchidae	*Ambloplites rupestris* *Centrarchus micropterus* *Enneacanthus gloriosus* *Lepomis auritus* *Lepomis cyanellus* *Lepomis gibbosus* *Lepomis gulosus* *Lepomis macrochirus* *Lepomis microlophus* *Micropterus dolomieu* *Micropterus salmoides* *Pomoxis annularis* *Pomoxis nigromaculatus*	Rockbass Flier Bluespotted sunfish Redbreast sunfish Green sunfish Pumpkinseed Warmouth Bluegill Redear sunfish Smallmouth bass Largemouth bass White crappie Black crappie
Percidae	*Etheostoma olmstedi* *Perca flavescens* *Percina peltata* *Percina roanoka* *Sandervitreus*	Tessellated darter Yellow perch Shield darter Roanoke darter Walleye
Scianidae	*Leiostomus xanthurus* *Micropogonias undulatus*	Spot Atlantic croaker
Achiriae	*Trinectes maculatus*	Hogchoker

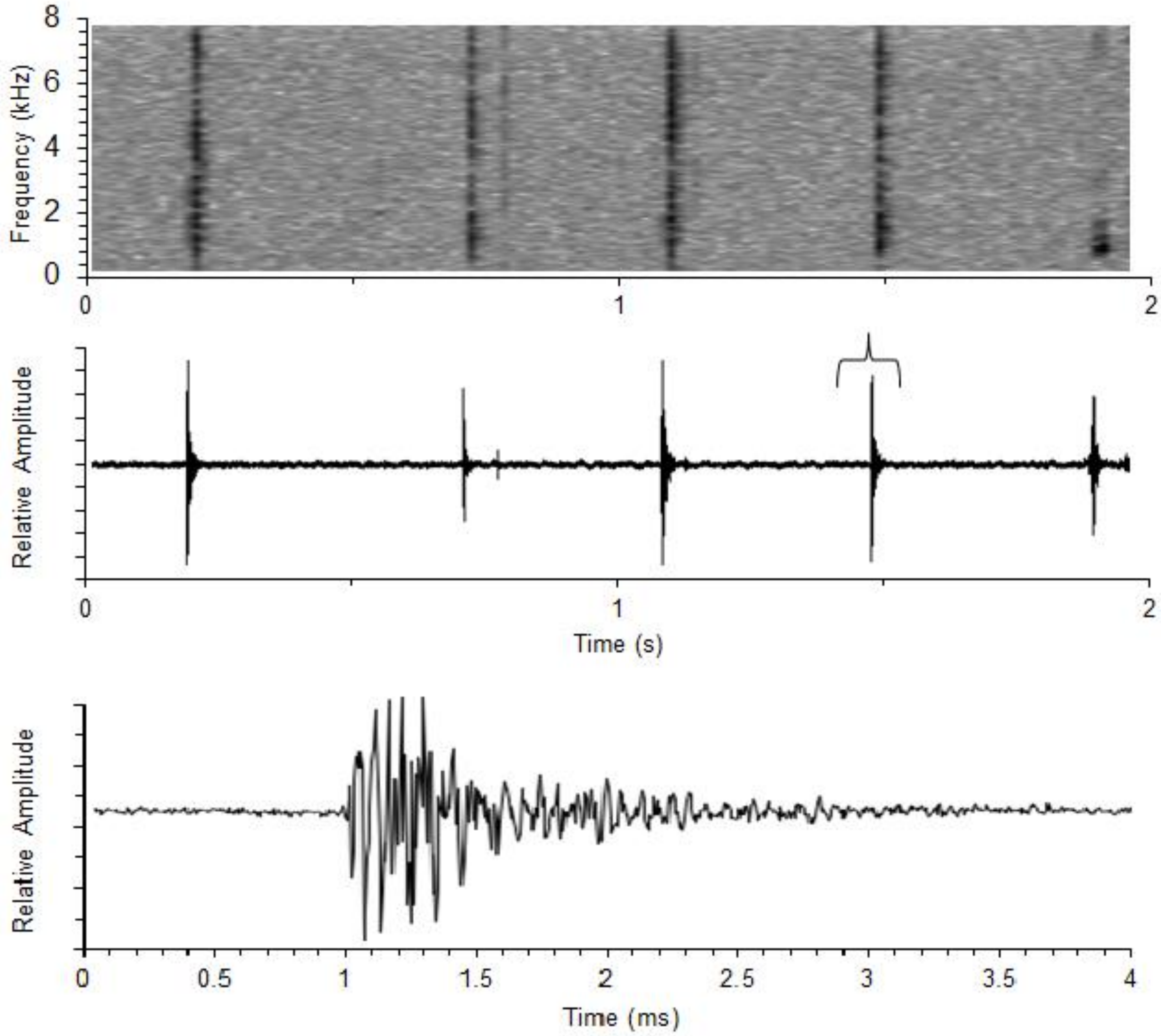

Figure 1. Spectogram and oscillogram for Clicks: close-up of waveform as indicated by bracket is shown on bottom graph.

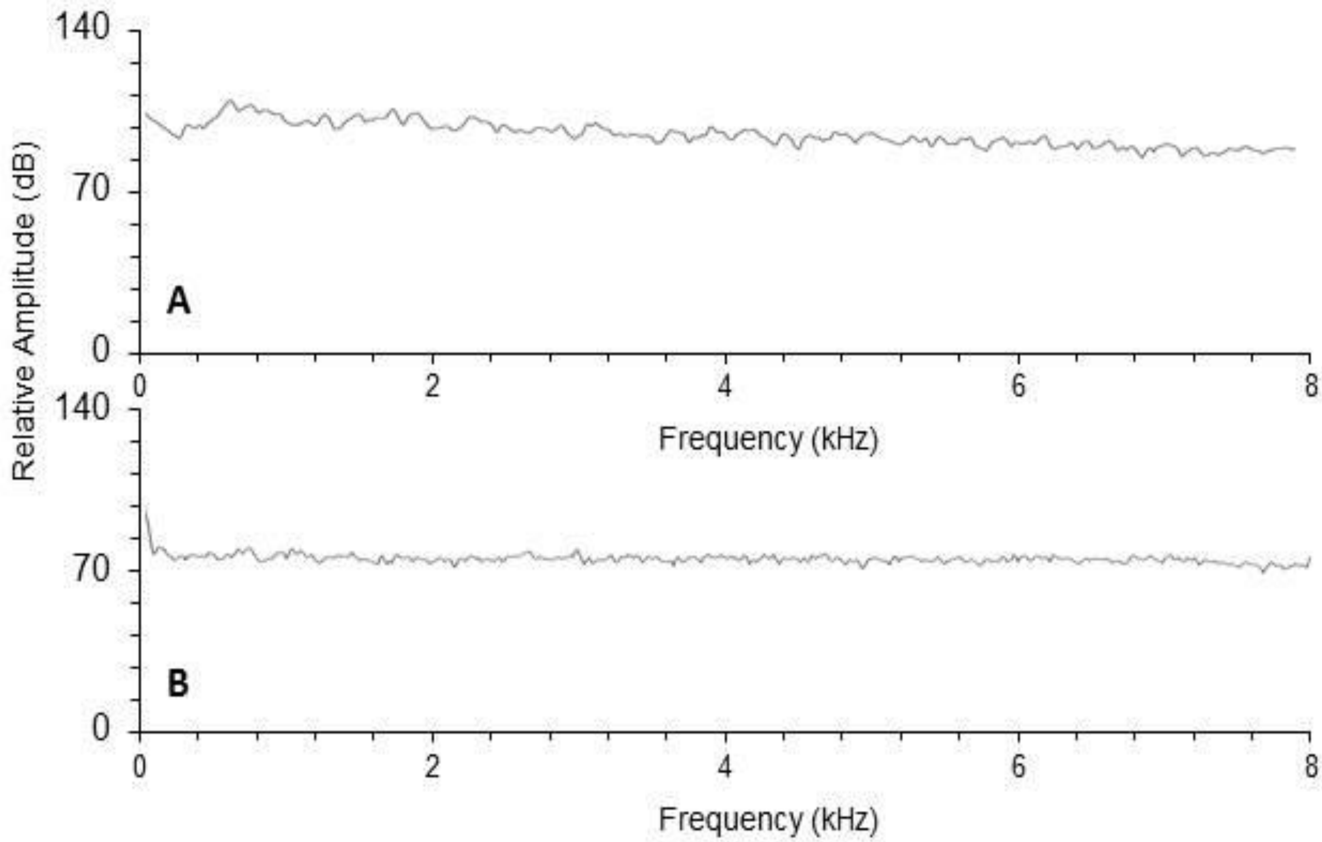

Figure 2. Power spectrum of Clicks (A) and background noise (B).Note that A was run with 10 clicks in succession to run at a higher sampling rate (940).

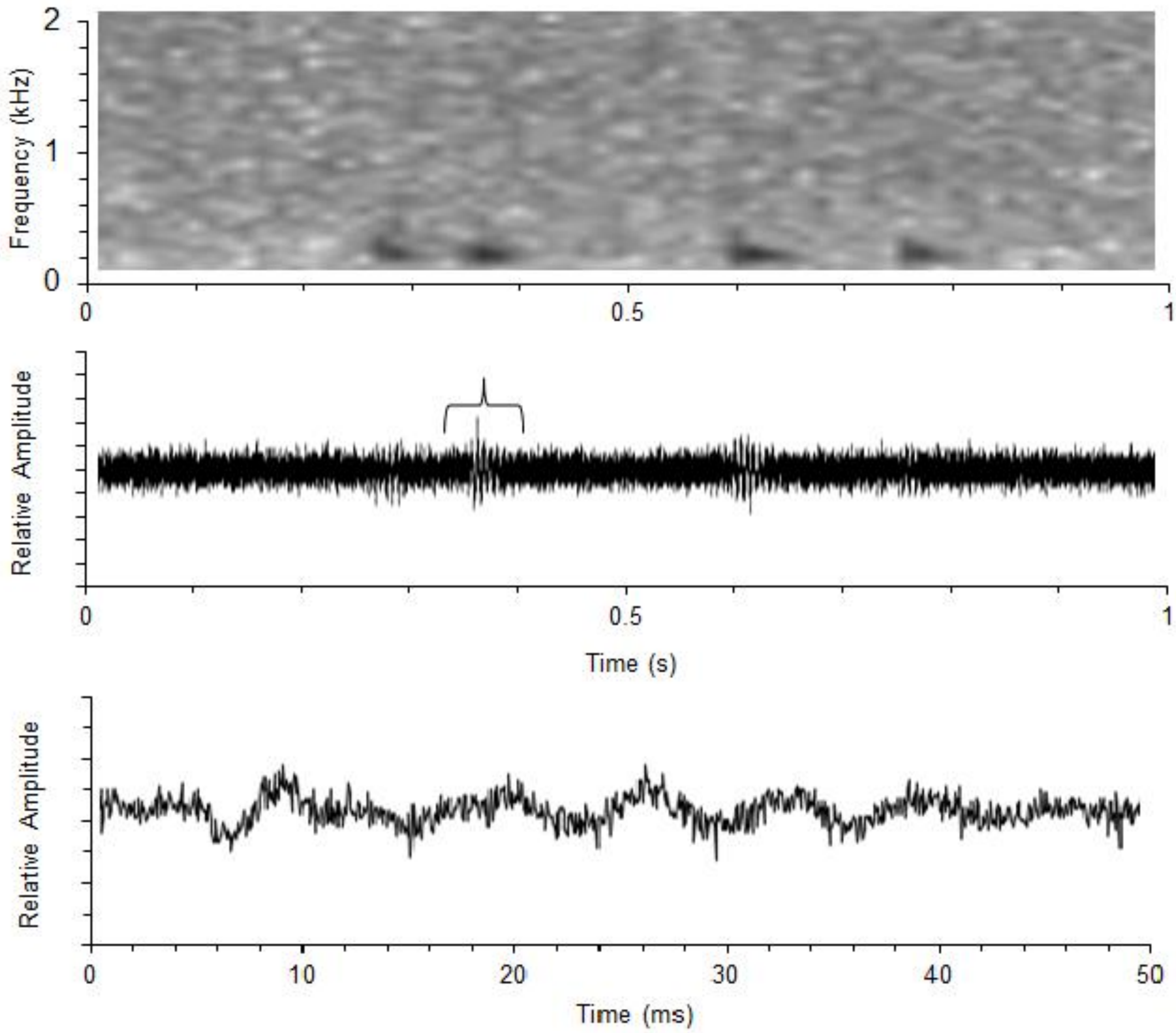

Figure 3. Spectogram and oscillogram for Runs: close-up of waveform as indicated by bracket is shown on bottom graph.

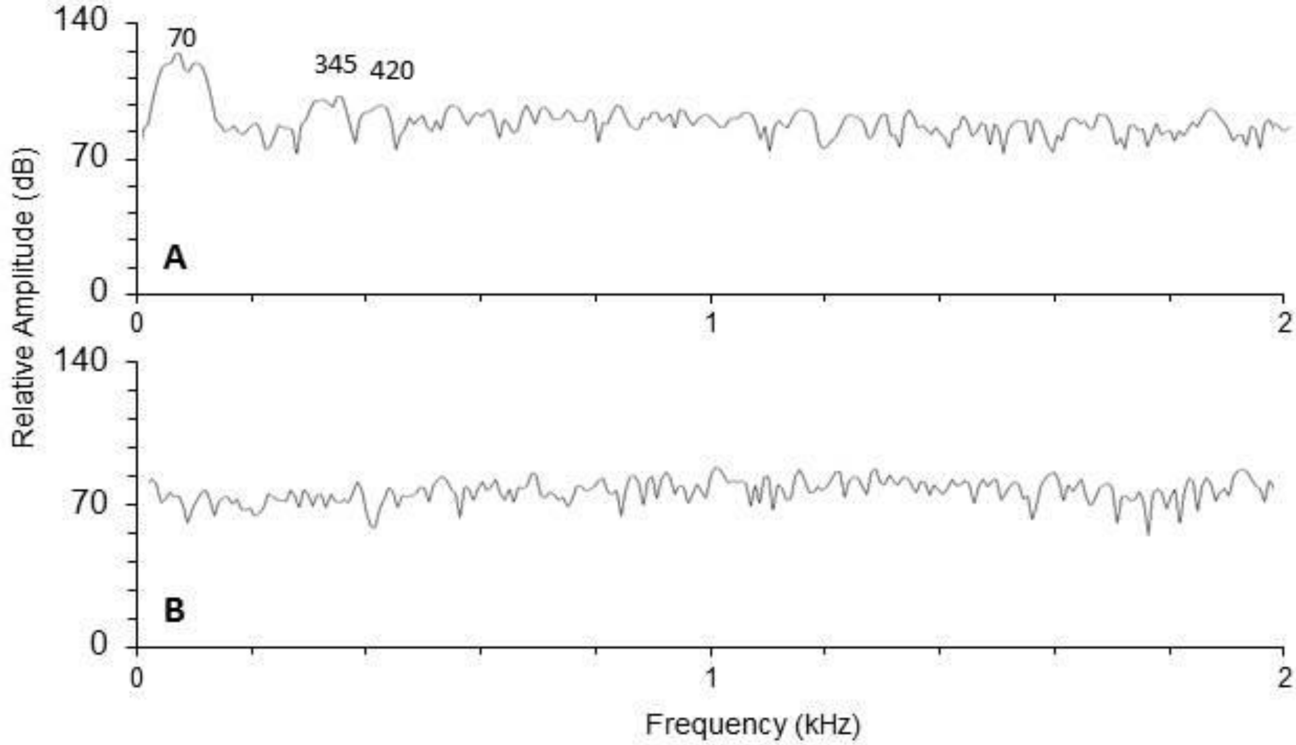

Figure 4. Power spectrum of Runs A) and background noise (B).Highest observed amplitude frequencies are marked on A (Hz).

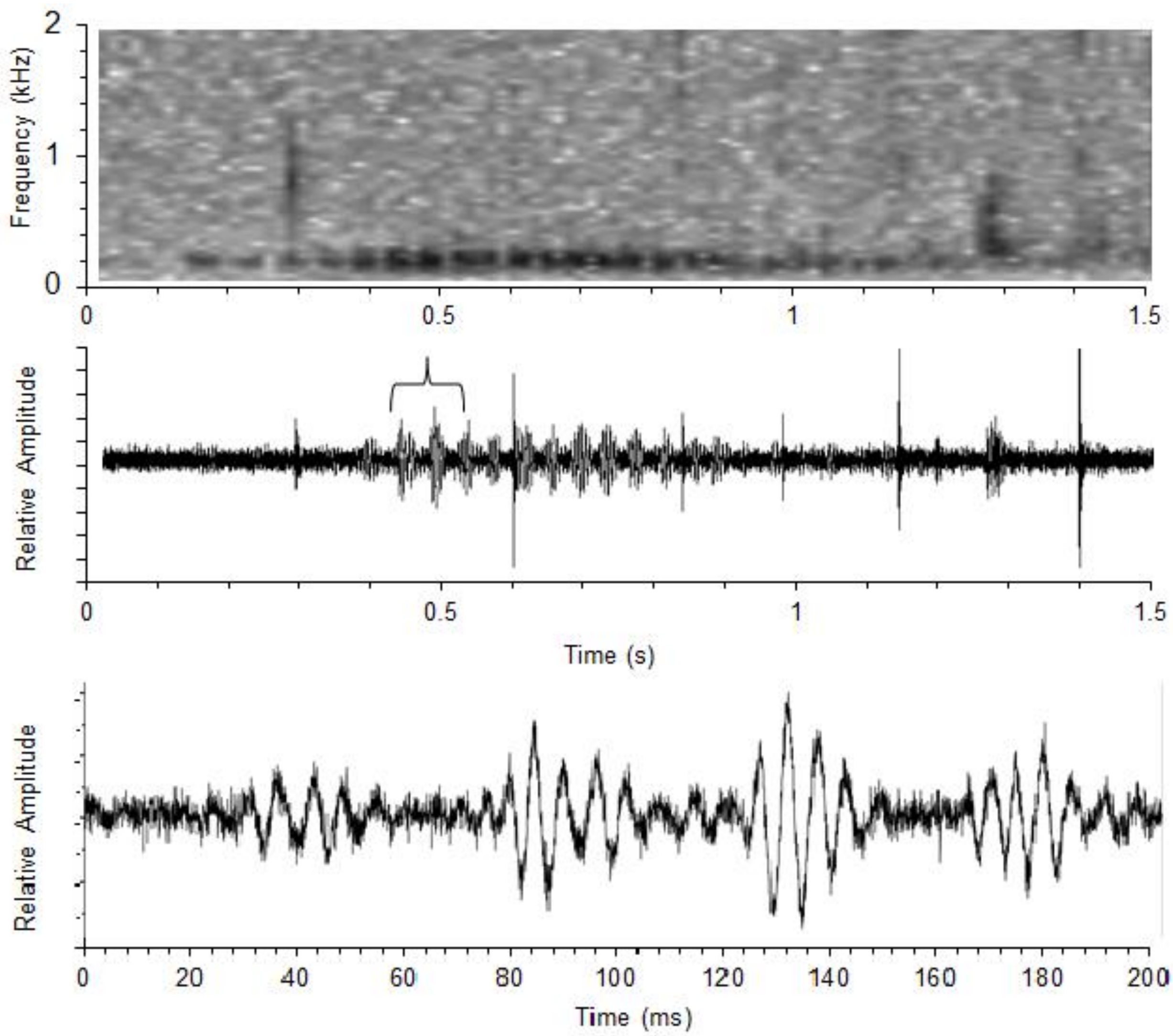

Figure 5. Spectogram and oscillogram for Croakss: close-up of waveform as indicated by bracket is shown on bottom graph. Note that high frequency short pulses are not part of croak. They are clicks.

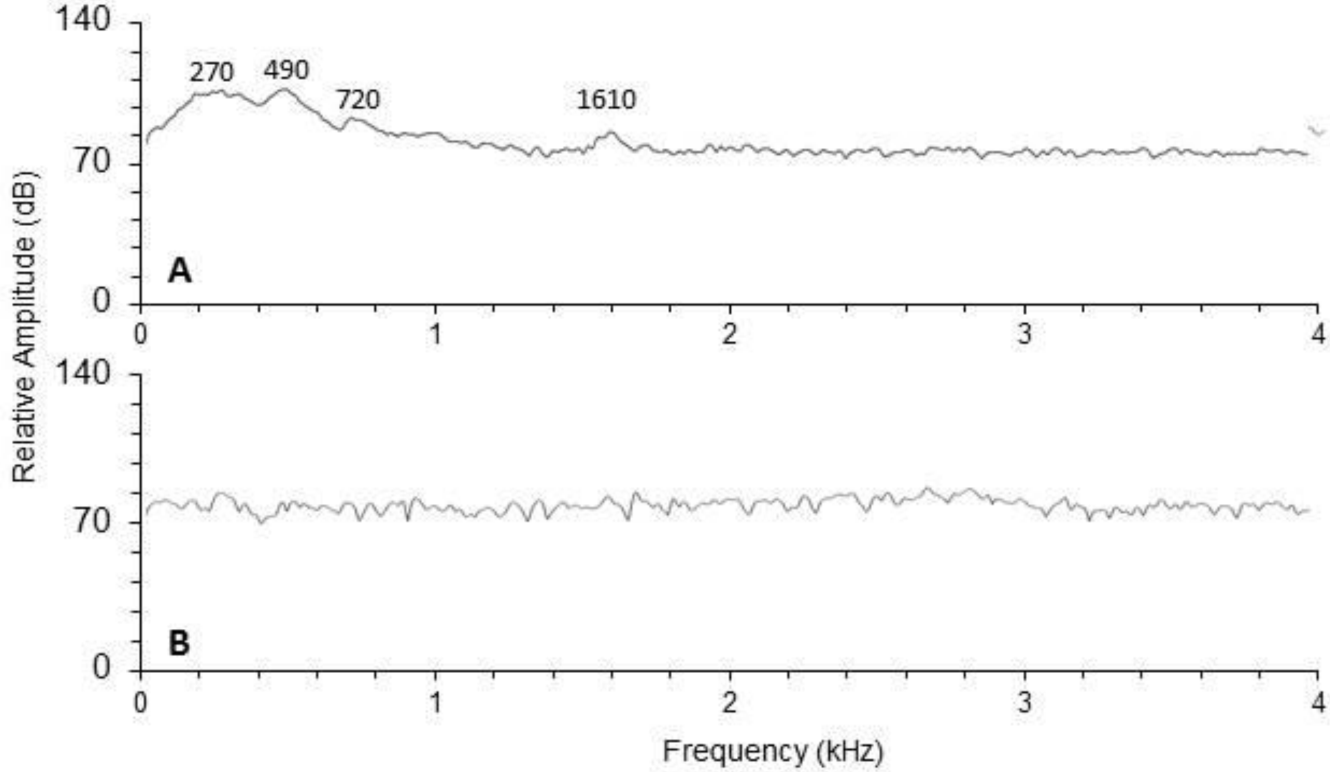

Figure 6. FFT of Croaks (A) and Background (B). Highest observed amplitude frequencies are marked on A (Hz).

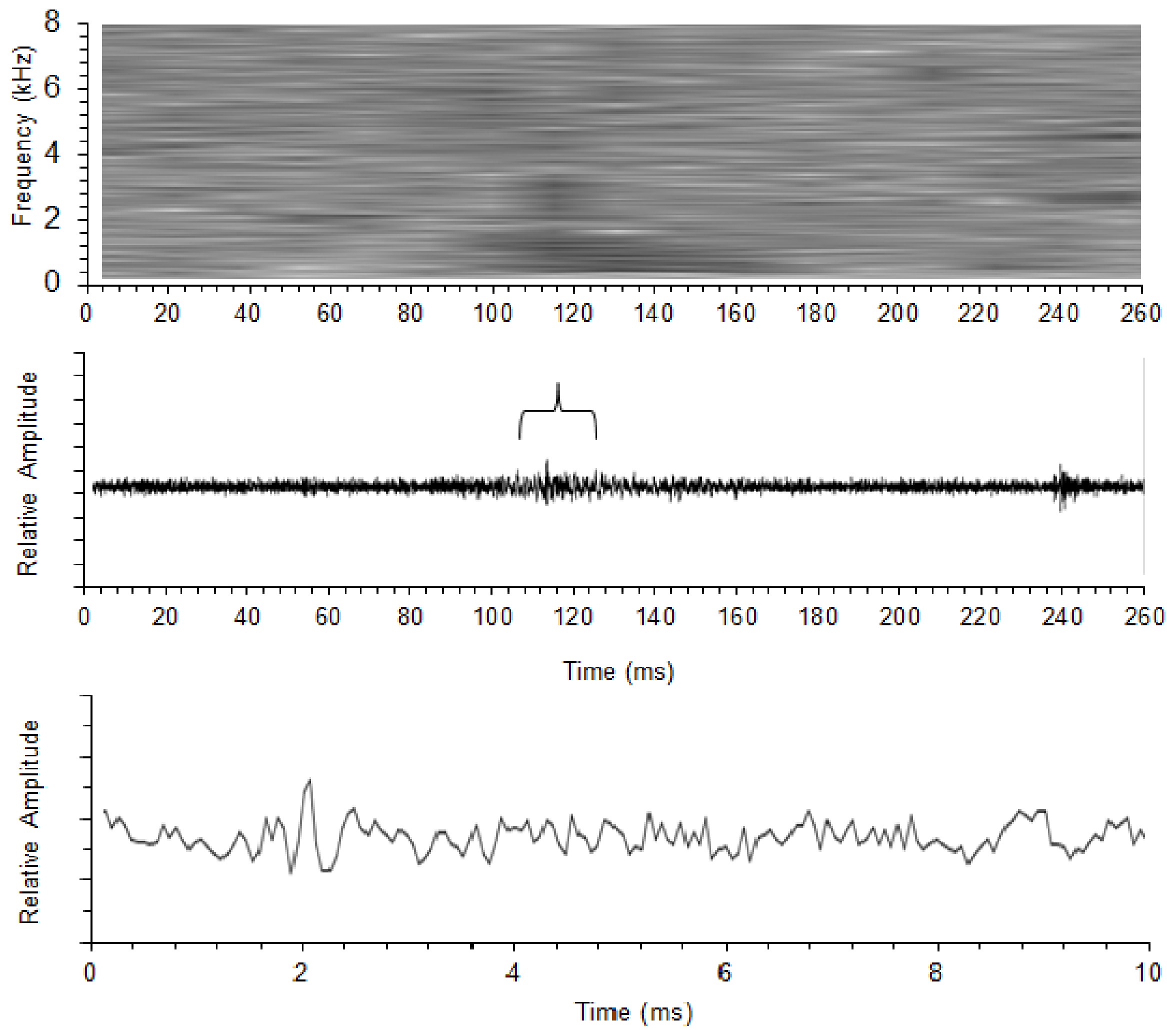

Figure 7. Spectogram and oscillogram for Sound 1: close-up of waveform as indicated by bracket is shown on bottom graph.

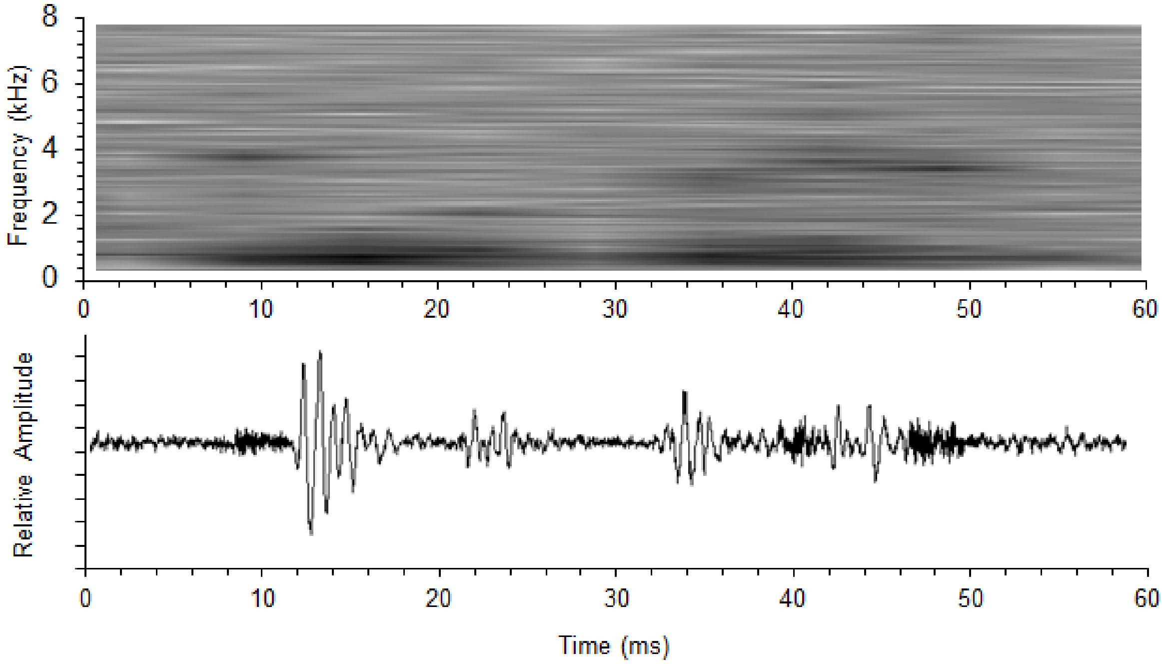

Figure 8. Spectogram and oscillogram for Sound 2.

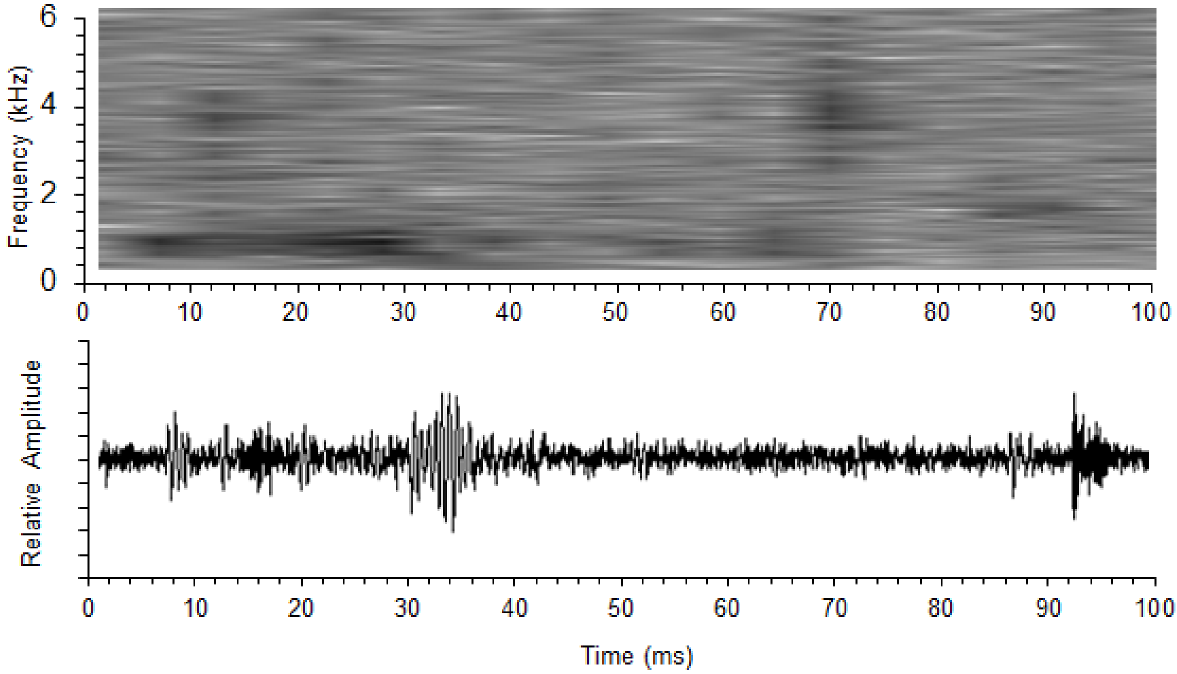

Figure 9. Spectogram and oscillogram for Sound 3

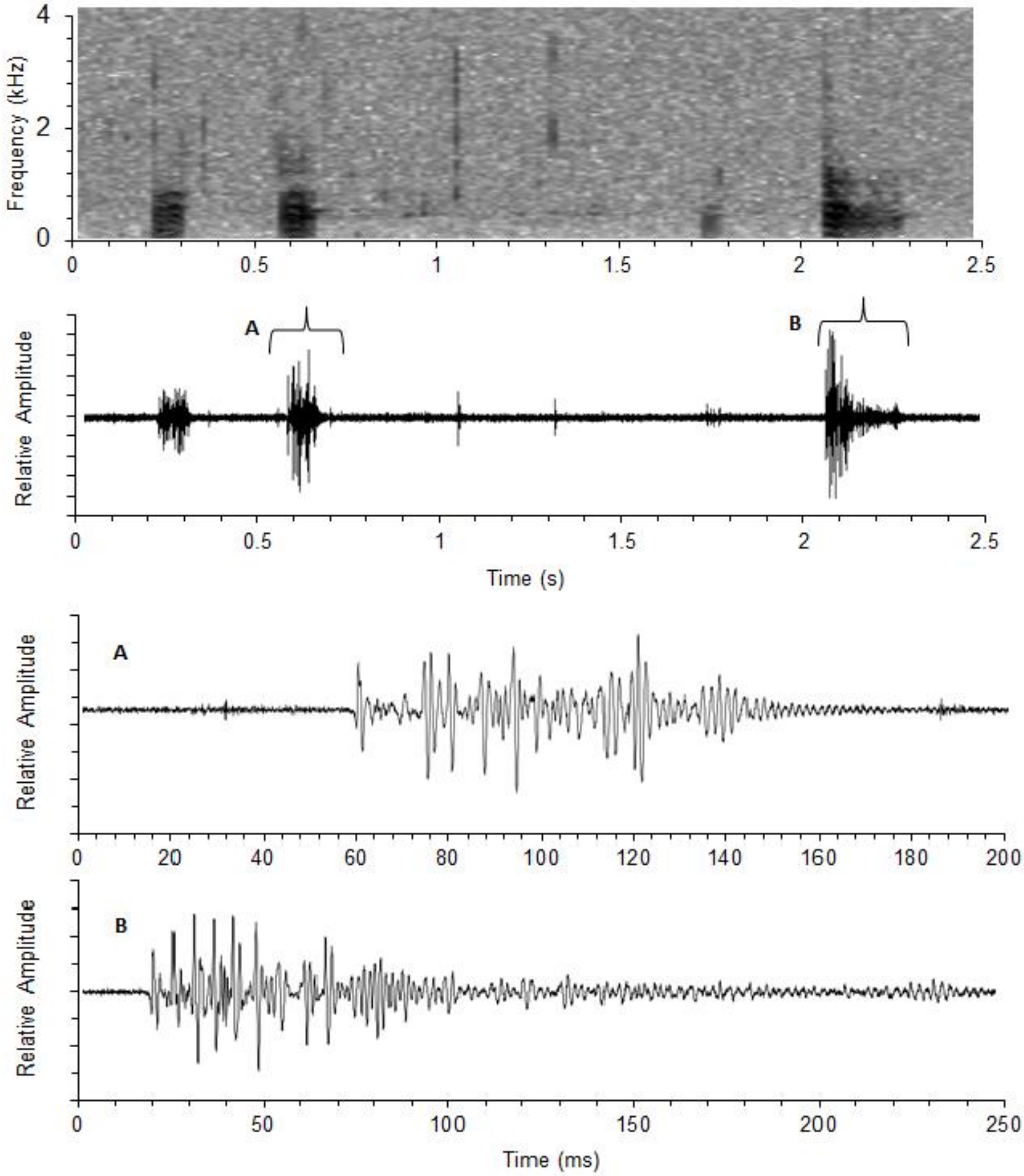

Figure 10. Spectogram and oscillogram for Sound 4: close-up of waveforms as indicated by brackets are shown on bottom graphs.

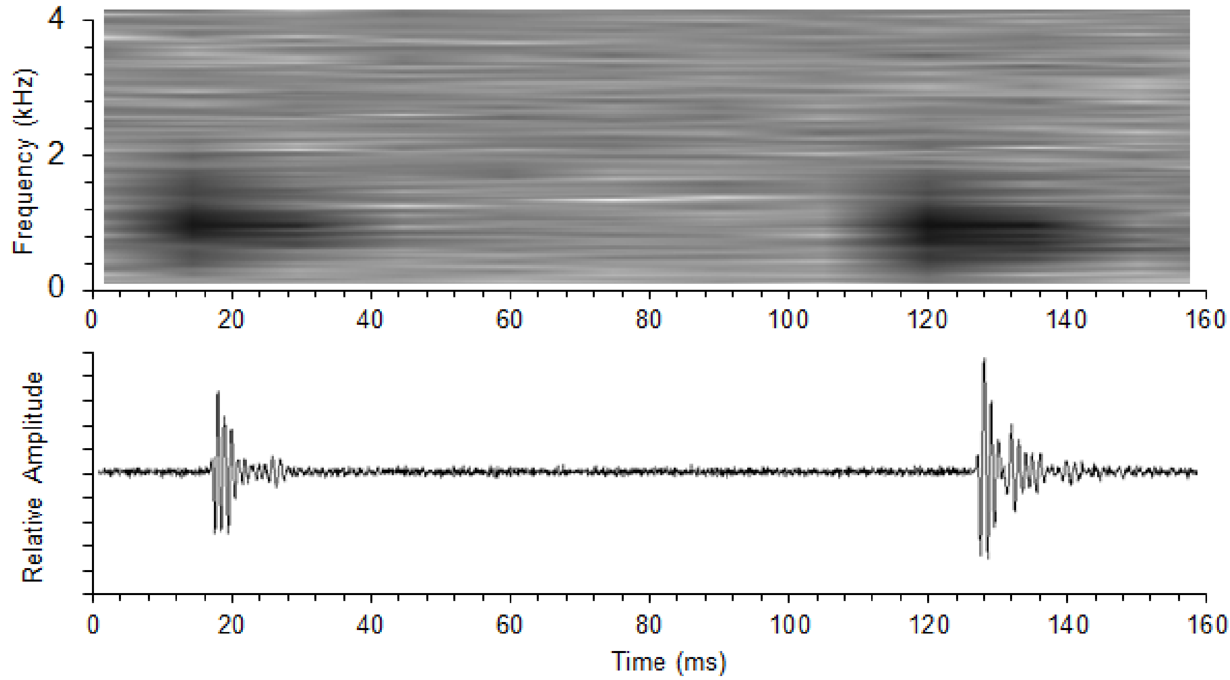

Figure 11. Spectogram and oscillogram for Sound 5

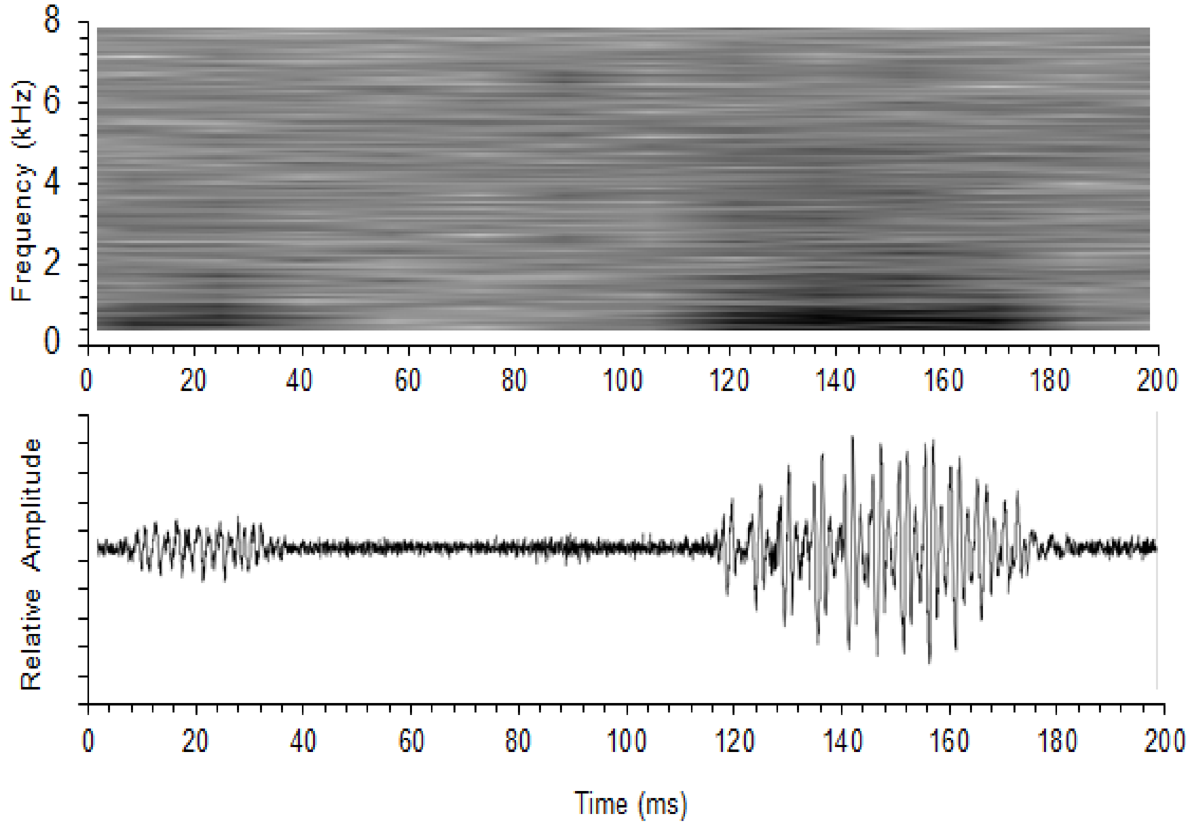

Figure 12. Spectogram and oscillogram for Sound 6.

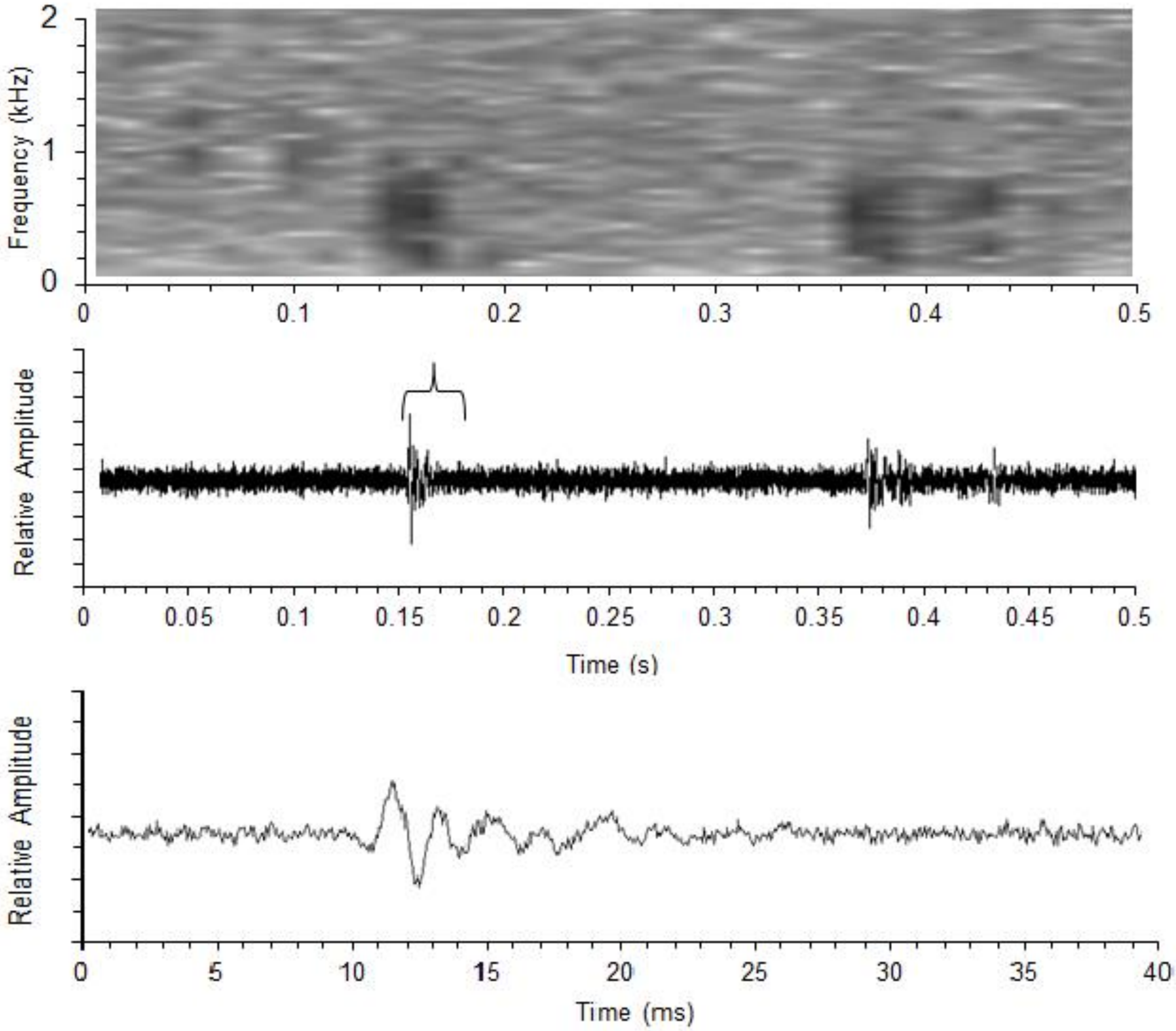

Figure 13. Spectogram and oscillogram for Sound 7: close-up of waveform as indicated by bracket is shown on bottom graph.

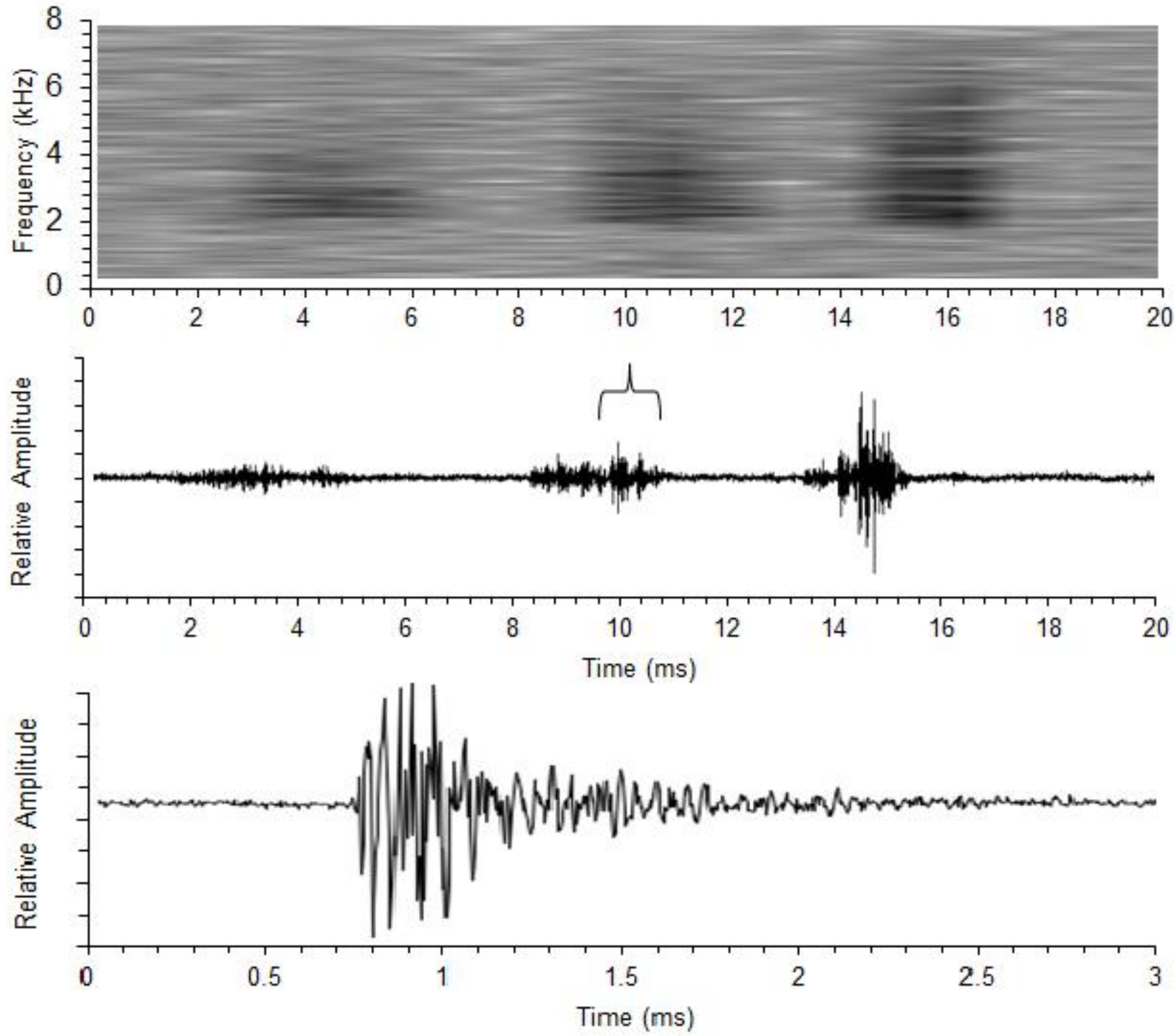

Figure 14. Spectogram and oscillogram for Sound 8: close-up of waveform as indicated by bracket is shown on bottom graph.

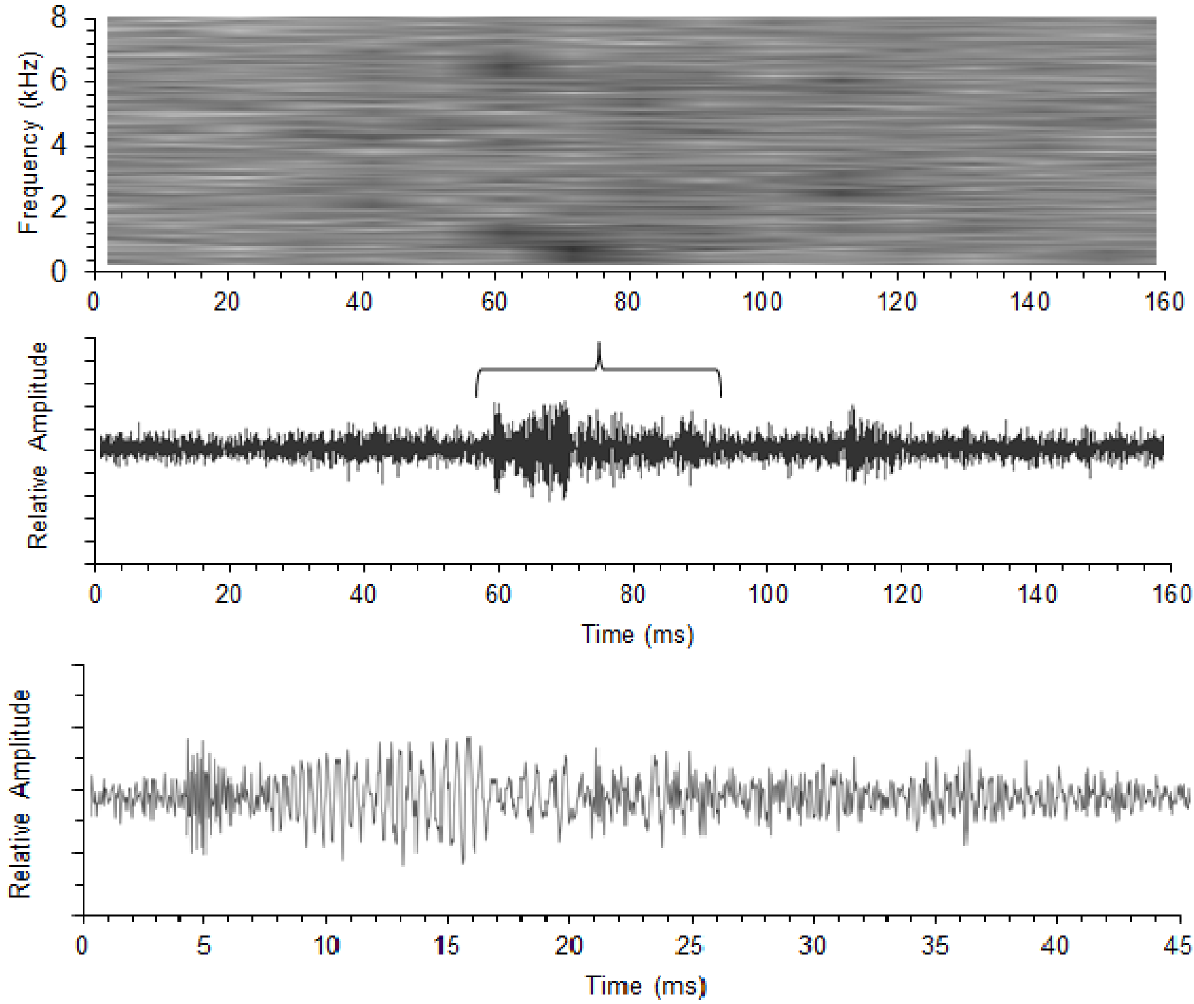

Figure 15. Spectogram and oscillogram for Sound 9: close-up of waveform as indicated by bracket is shown on bottom graph.

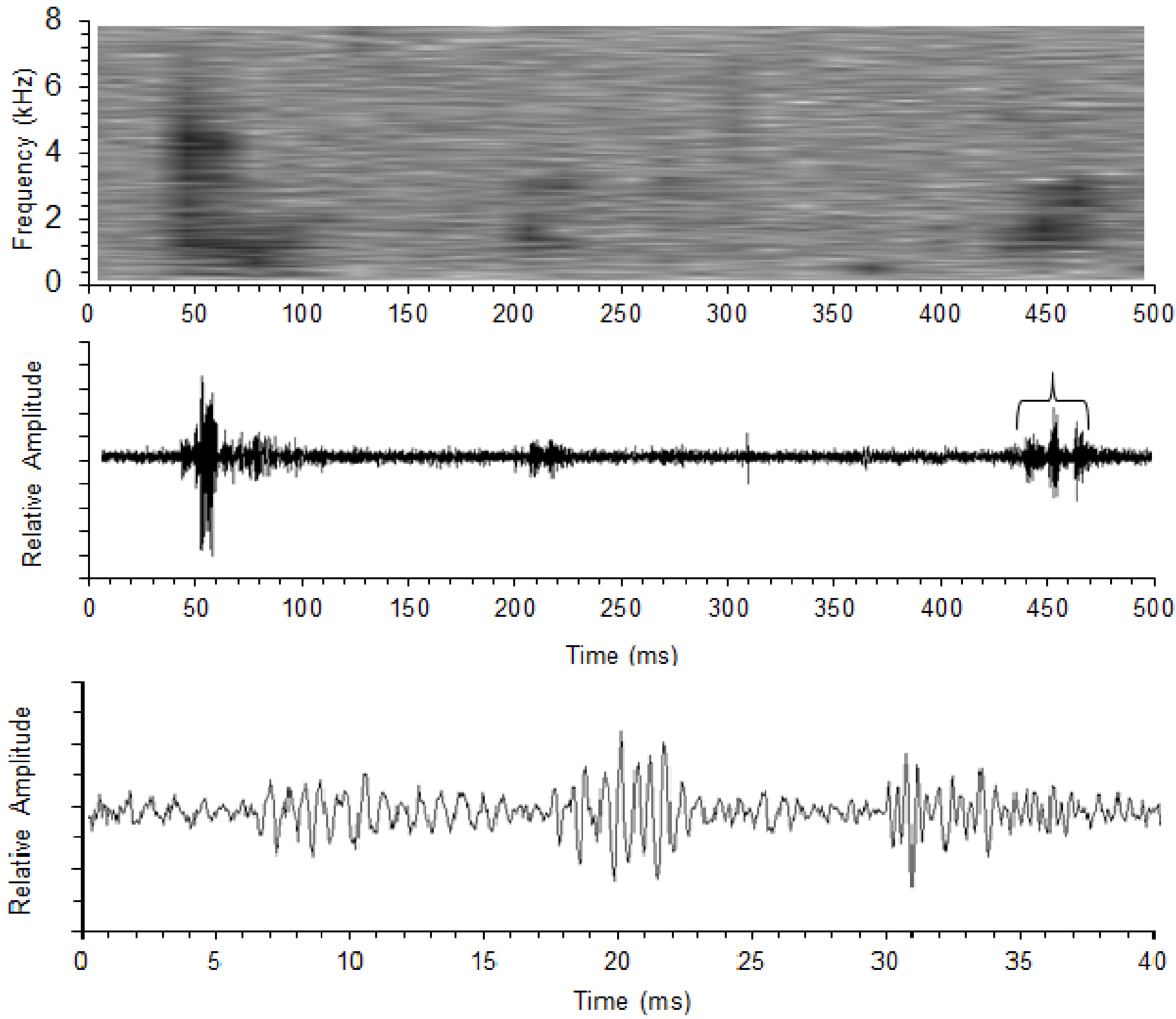

Figure 16. Spectogram and oscillogram for Sound 10: close-up of waveform as indicated by bracket is shown on bottom graph.

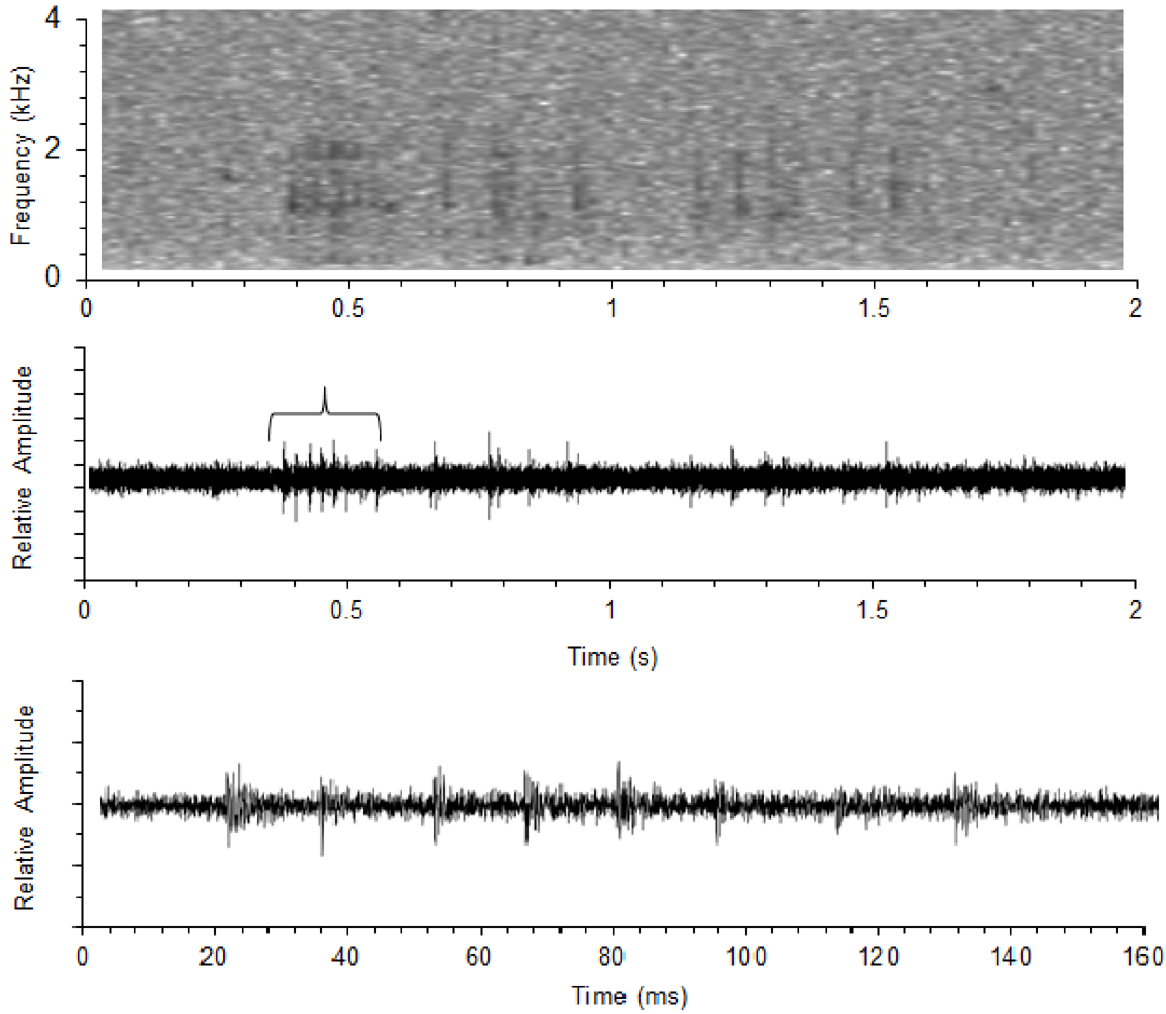

Figure 17. Spectogram and oscillogram for Sound 11: close-up of waveform as indicated by bracket is shown on bottom graph.

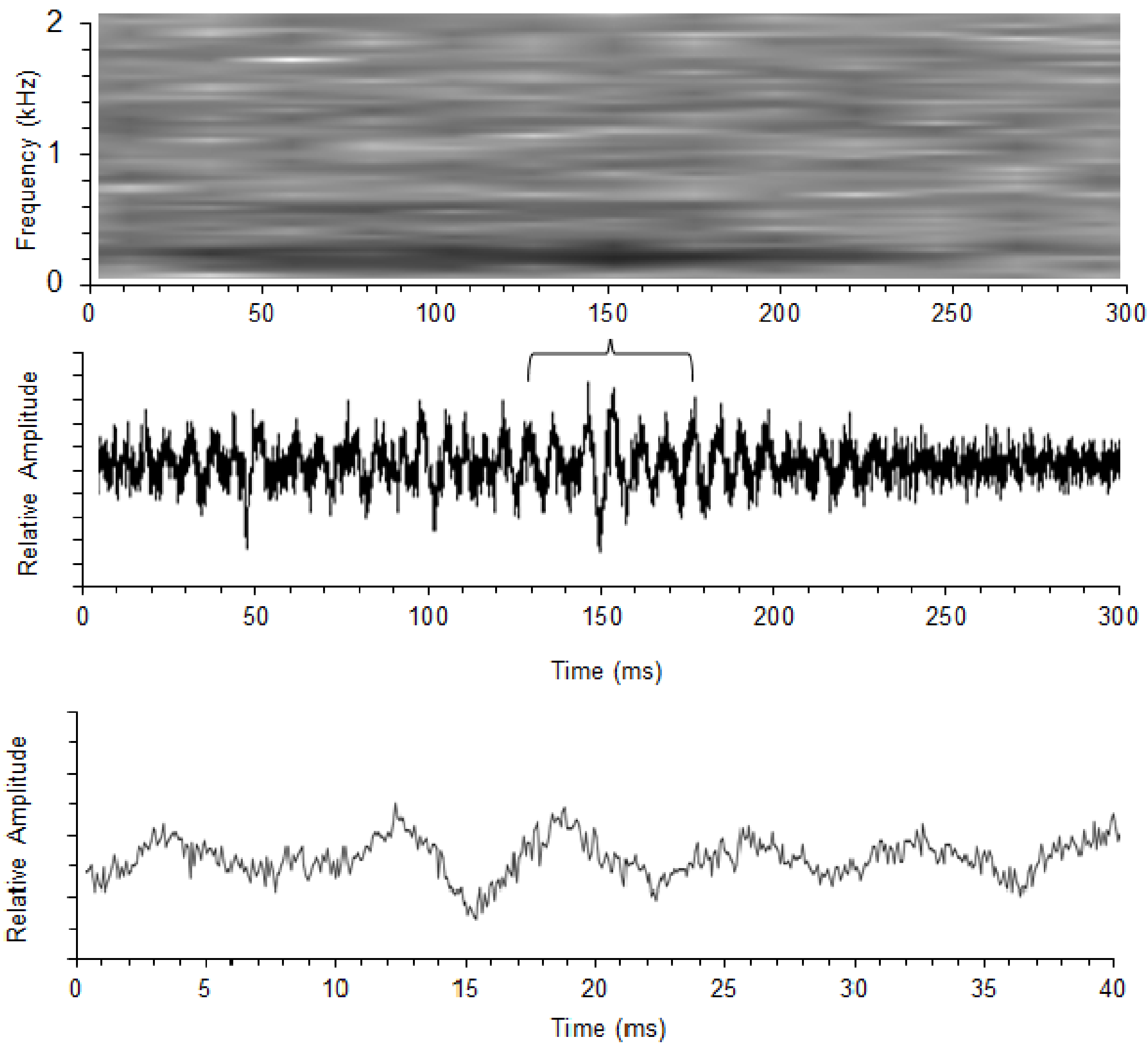

Figure 18. Spectogram and oscillogram for Sound 12: close-up of waveform as indicated by bracket is shown on bottom graph..

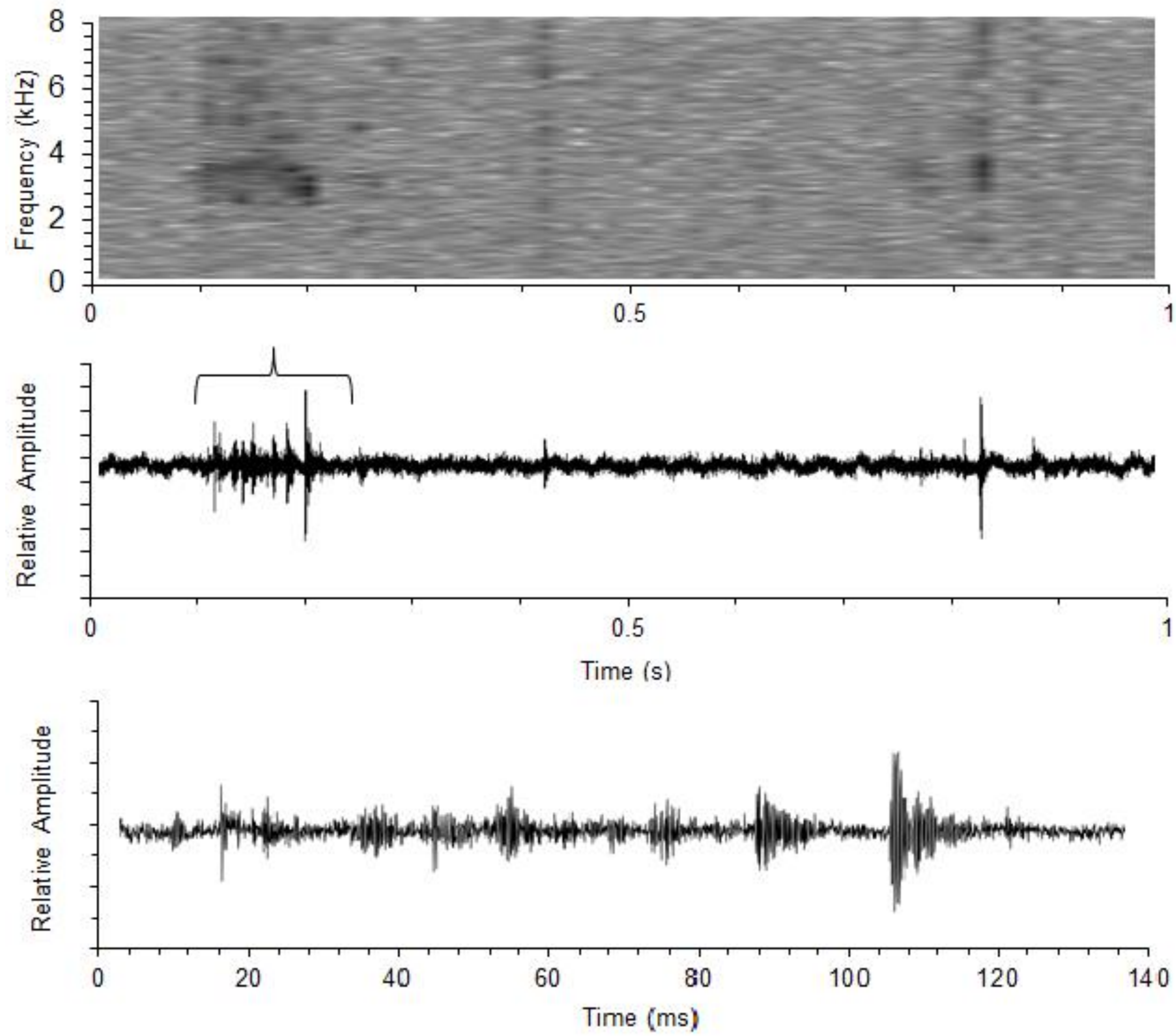

Figure 19. Spectogram and oscillogram for Sound 13: close-up of waveform as indicated by bracket is shown on bottom graph.

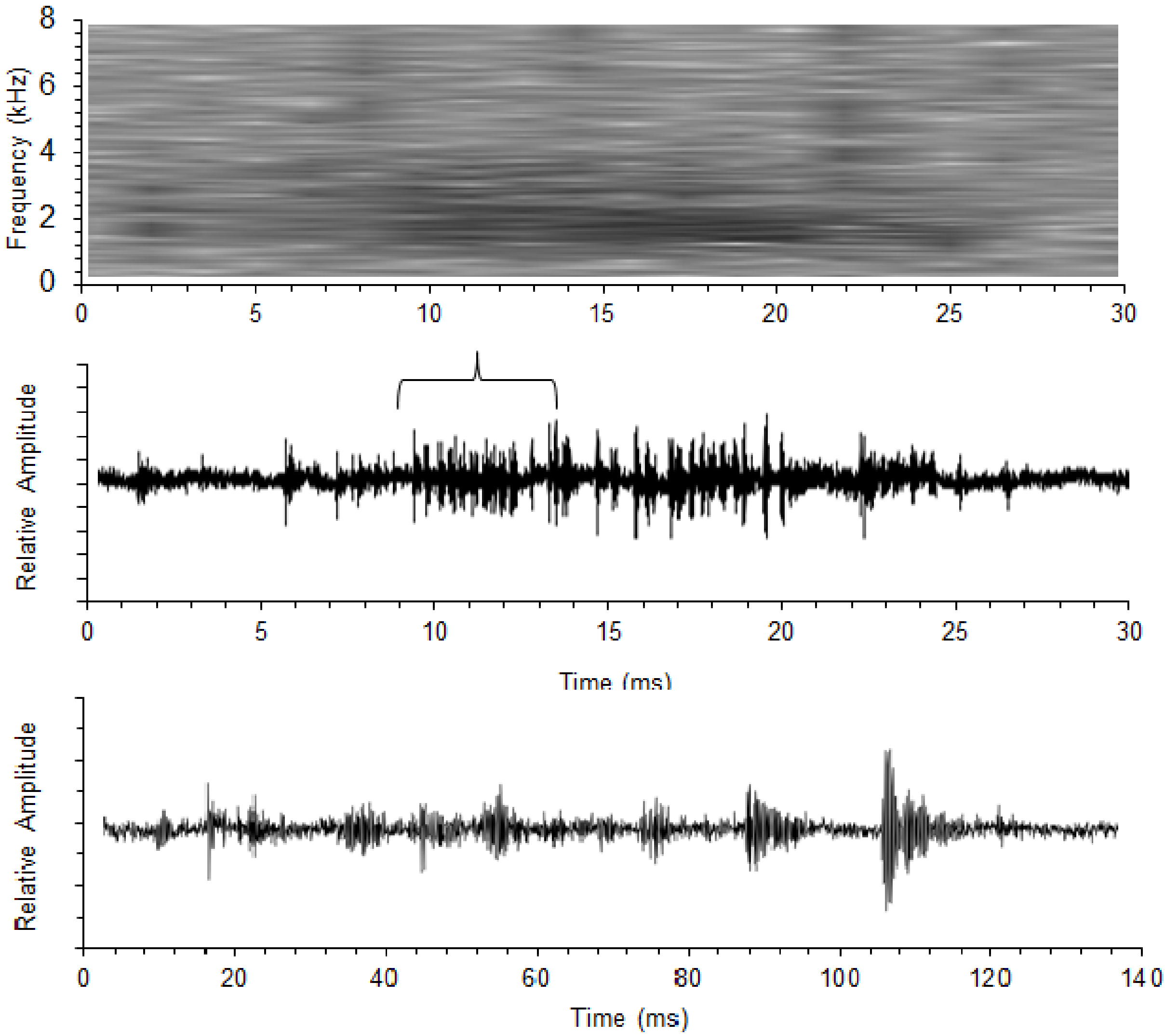

Figure 20. Spectogram and oscillogram for Sound 14: close-up of waveform as indicated by bracket is shown on bottom graph.

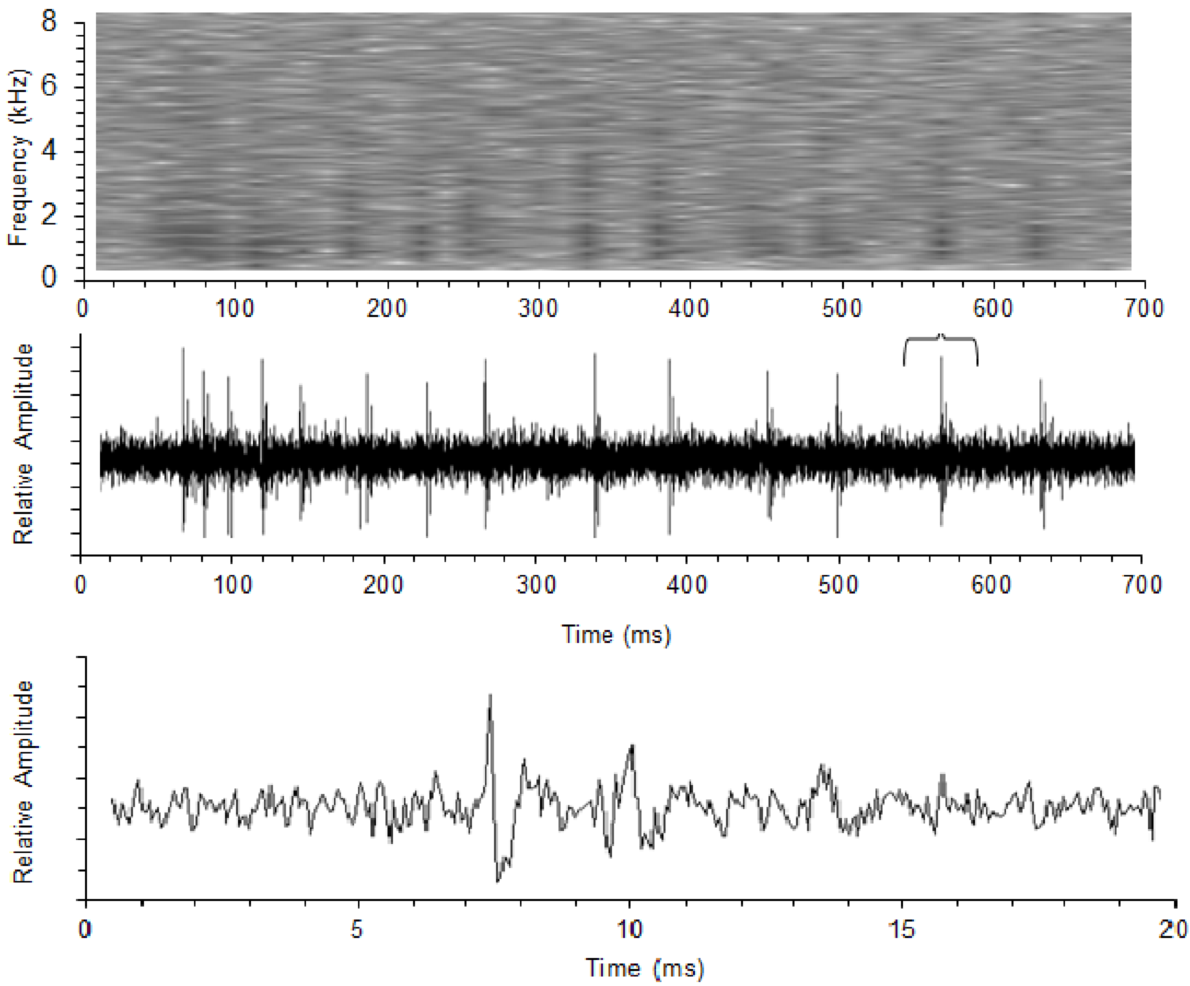

Figure 21. Spectogram and oscillogram for Sound 15: close-up of waveform as indicated by bracket is shown on bottom graph.

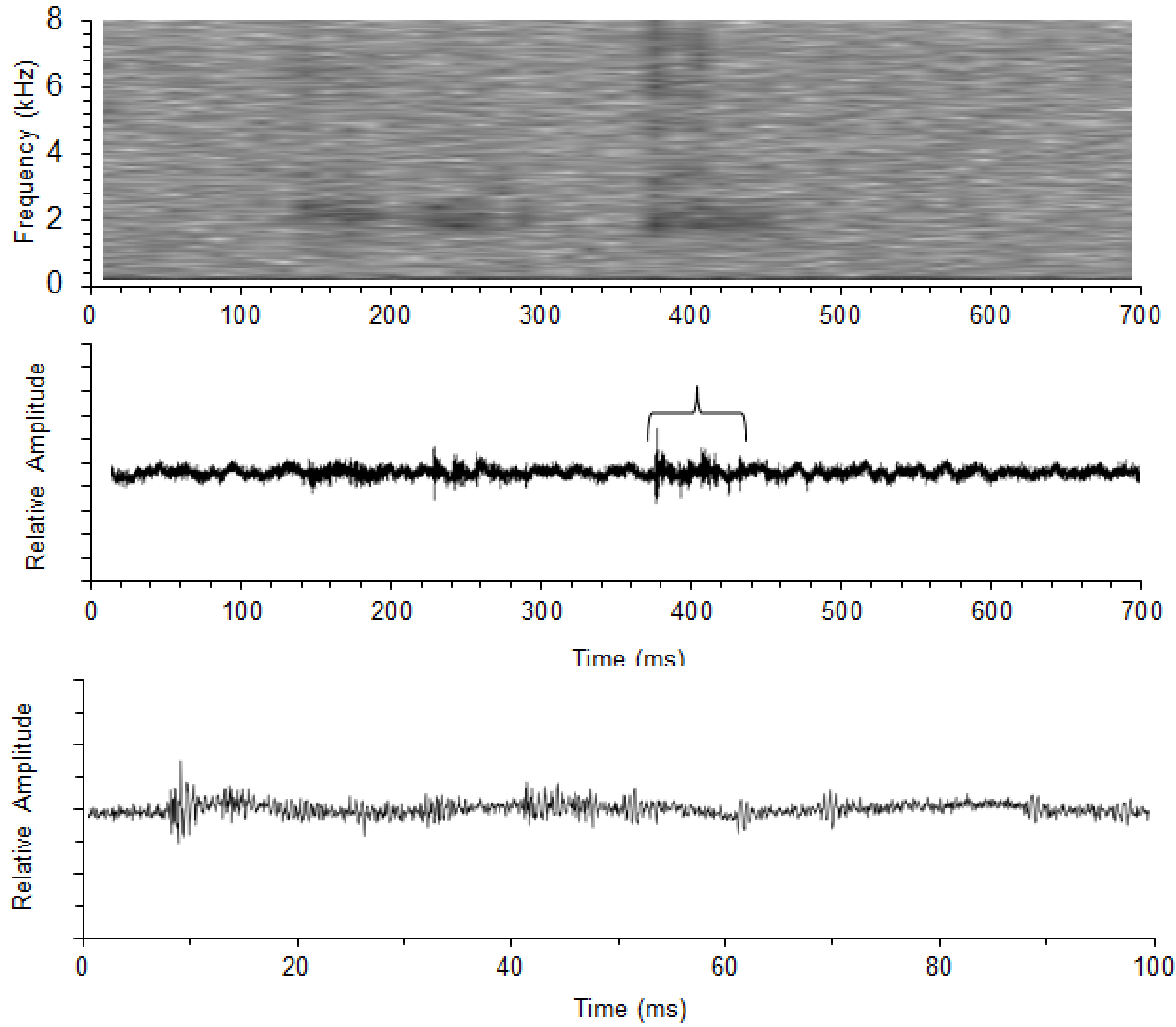

Figure 22. Spectogram and oscillogram for Sound 16: close-up of waveform as indicated by bracket is shown on bottom graph.

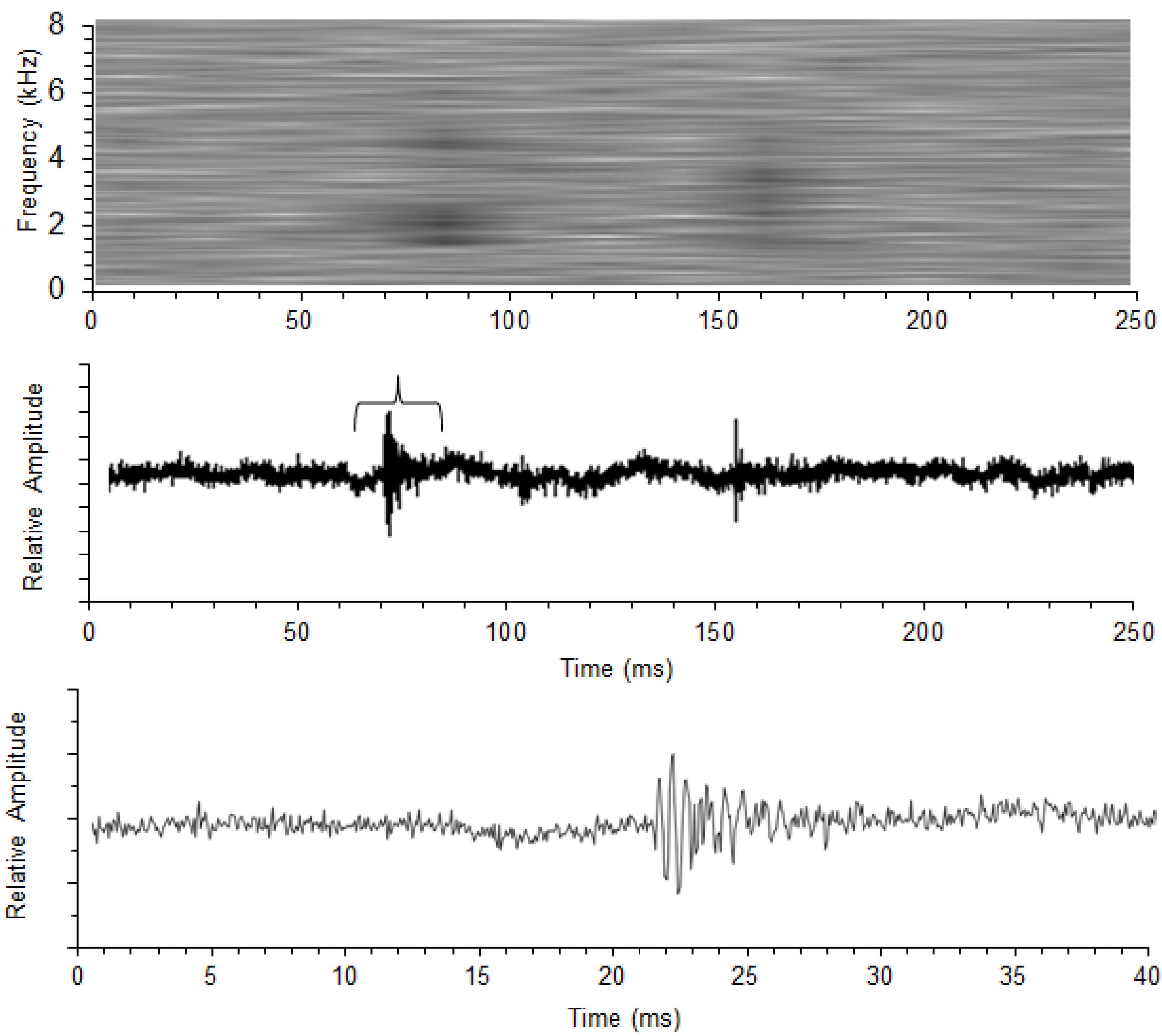

Figure 23. Spectogram and oscillogram for Sound 17: close-up of waveform as indicated by bracket is shown on bottom graph.

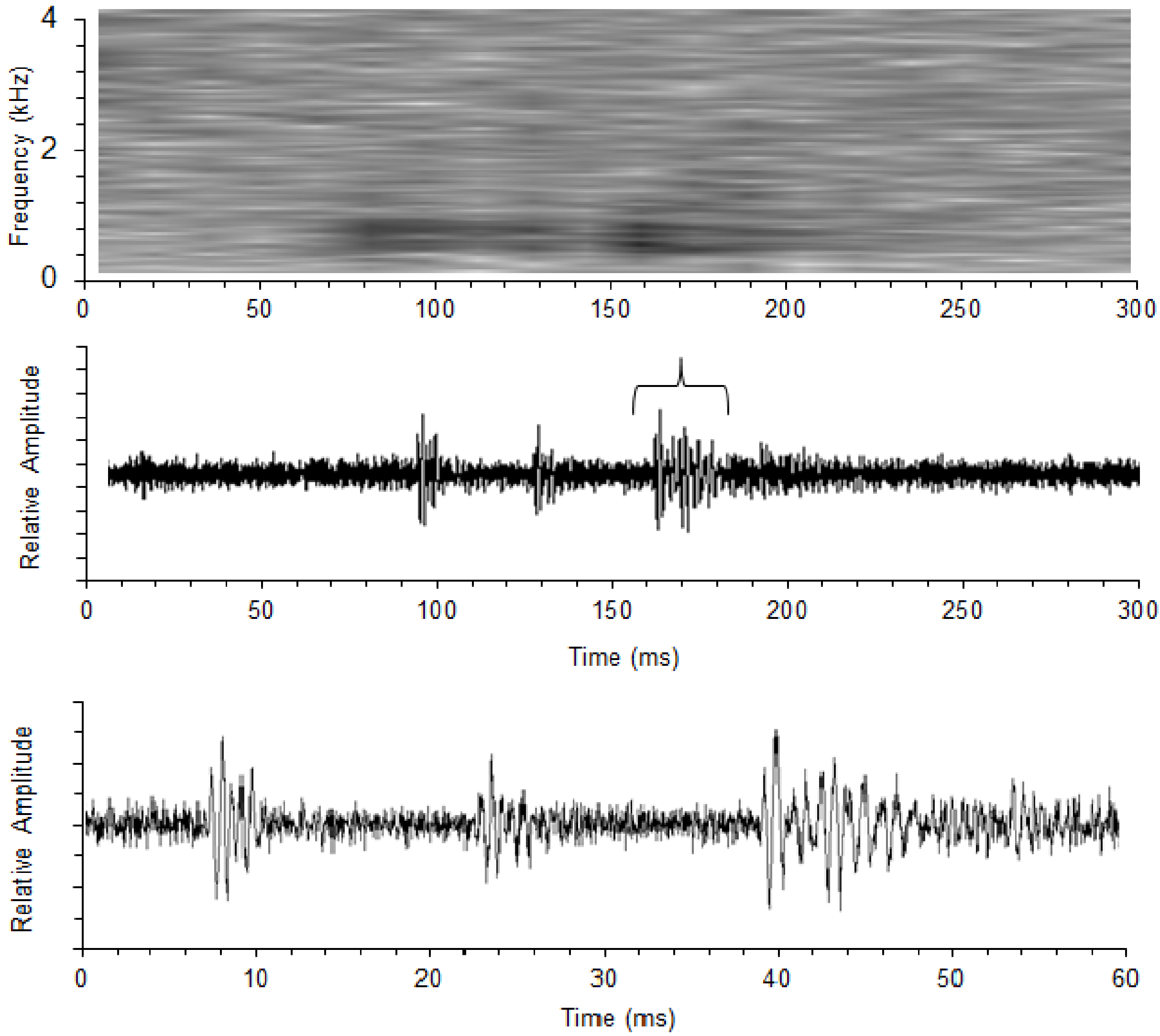

Figure 24. Spectogram and oscillogram for Sound 18: close-up of waveform as indicated by bracket is shown on bottom graph.

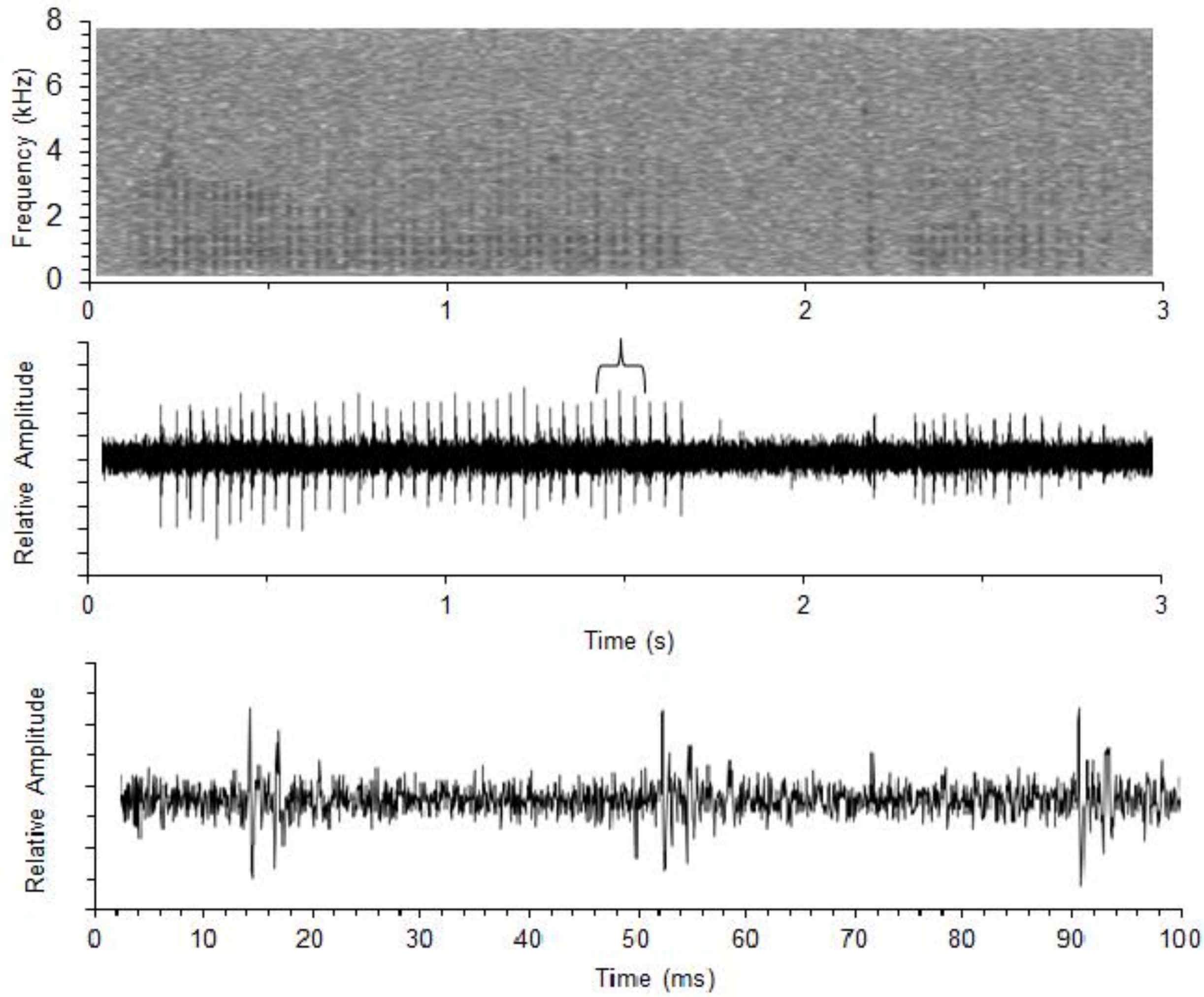

Figure 25. Spectogram and oscillogram for Sound 19: close-up of waveform as indicated by bracket is shown on bottom graph.

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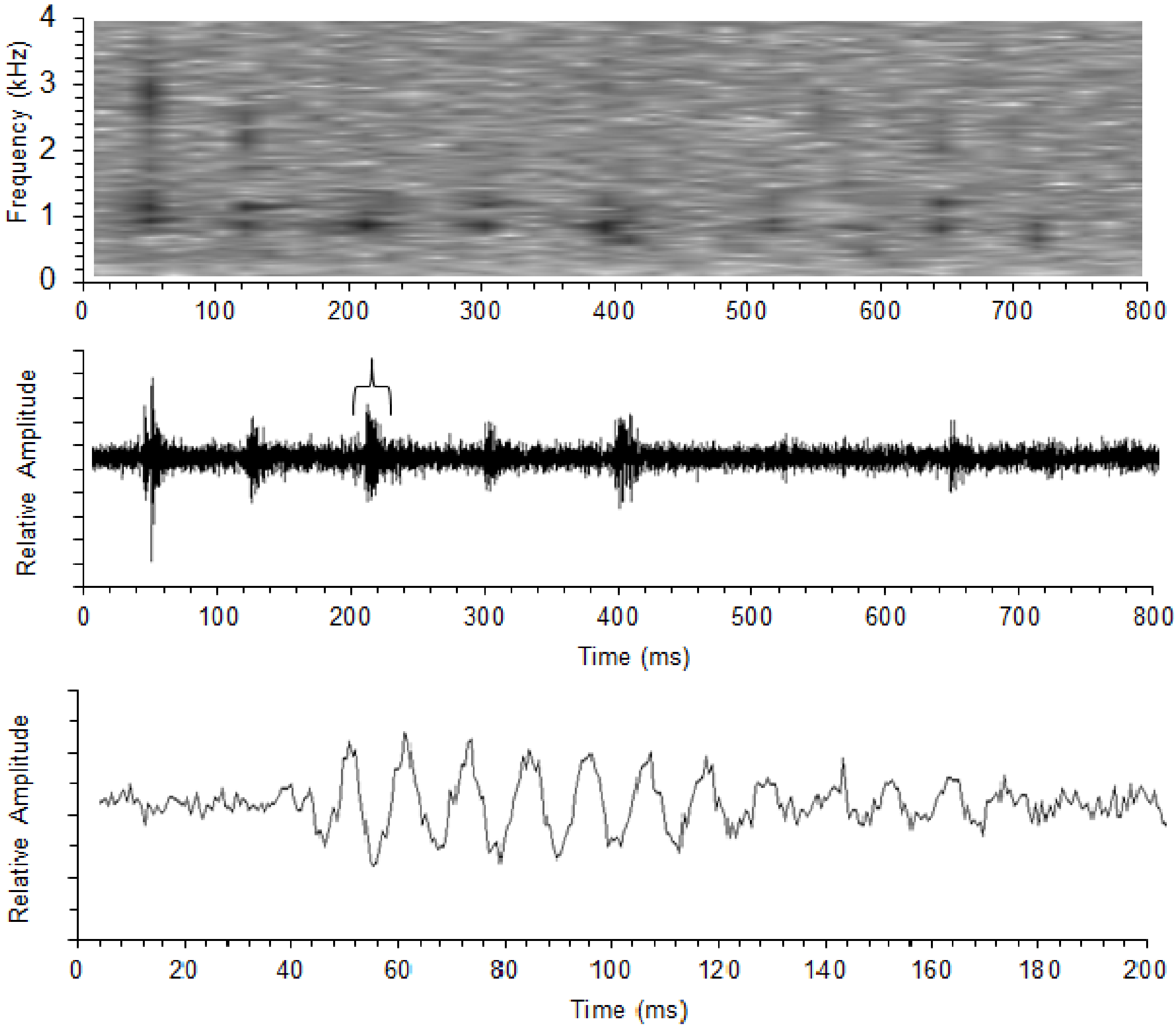

Figure 26. Spectogram and oscillogram for Sound 20: close-up of waveform as indicated by bracket is shown on bottom graph.

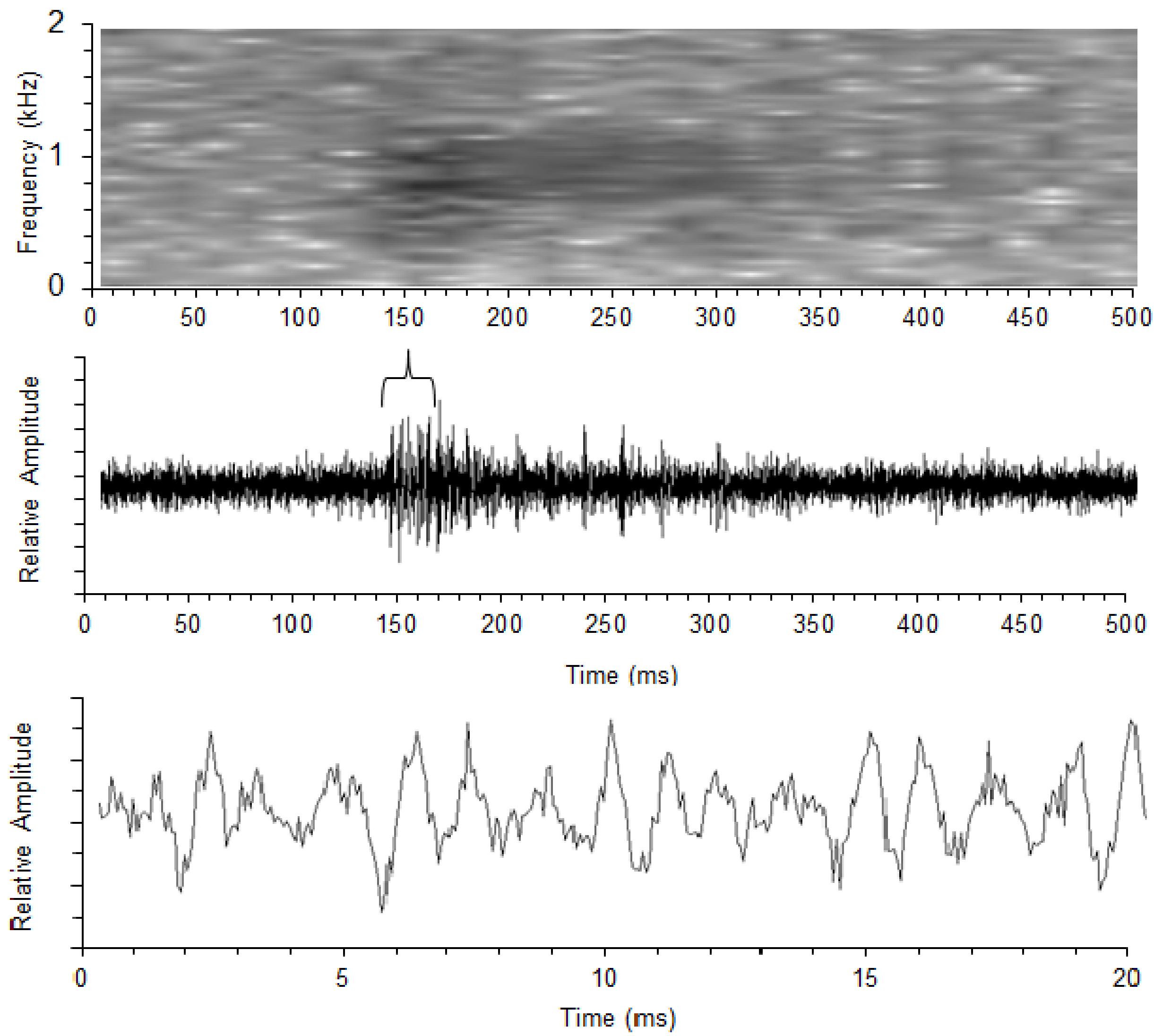

Figure 27. Spectogram and oscillogram for Sound 21: close-up of waveform as indicated by bracket is shown on bottom graph.

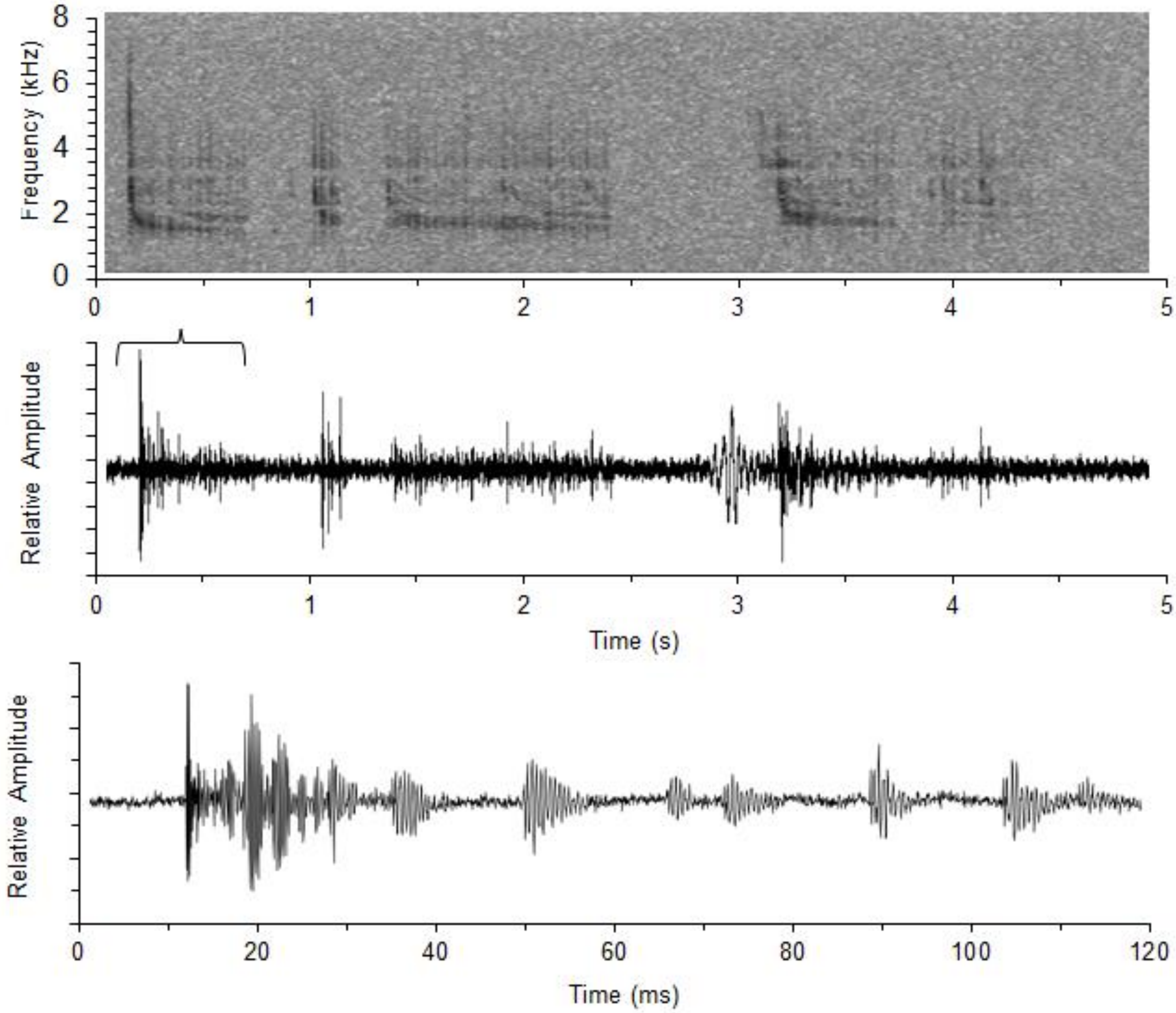

Figure 28. Spectogram and Oscillogram for Sound 22, Close-up of waveform as indicated by bracket is shown on bottom graph.

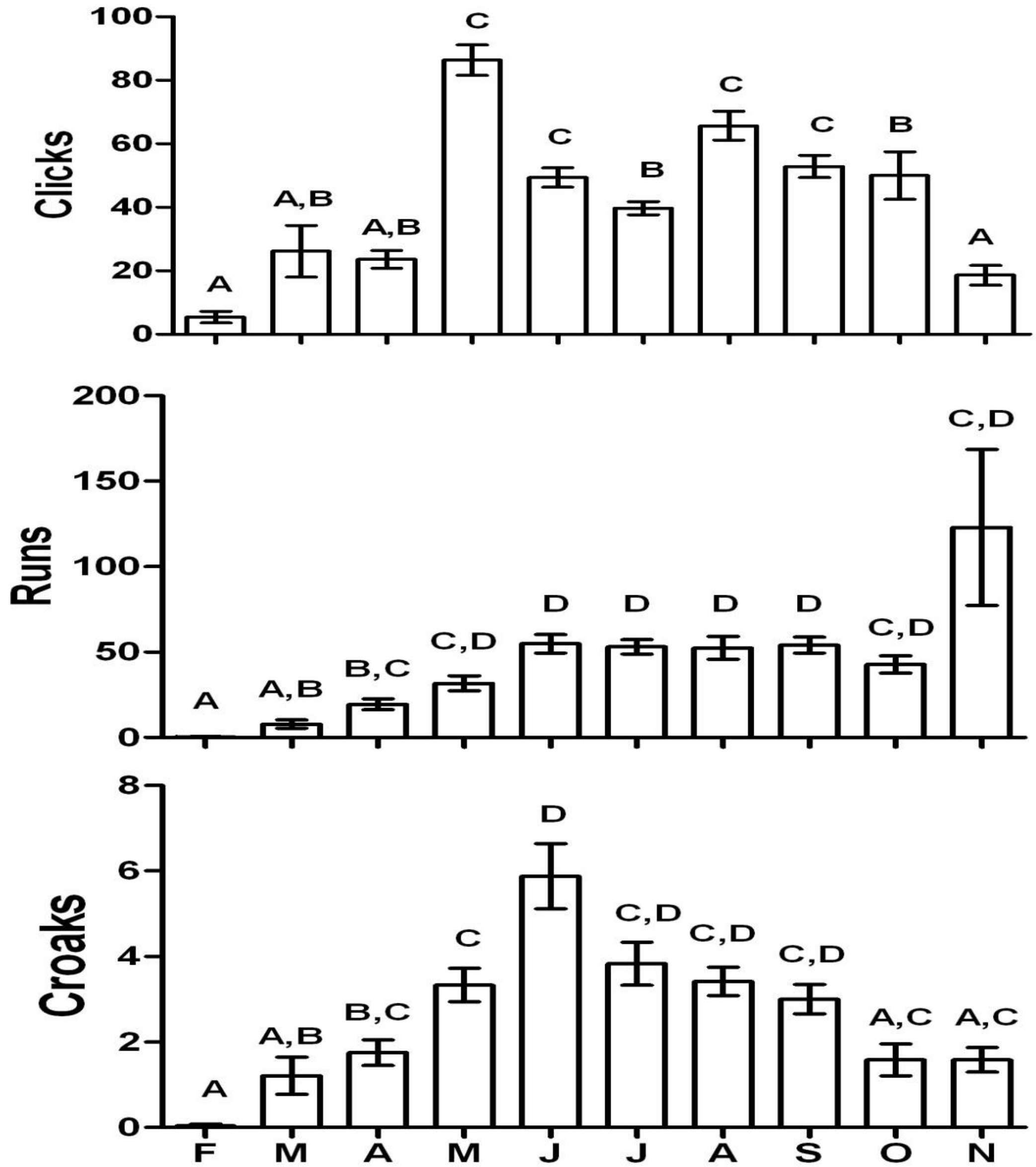

Figure 29. Mean ± SE monthly occurrences of clicks, runs, and croaks. Groups marked with the same letter are statistically similar, and groups with differing letters are statistically different.

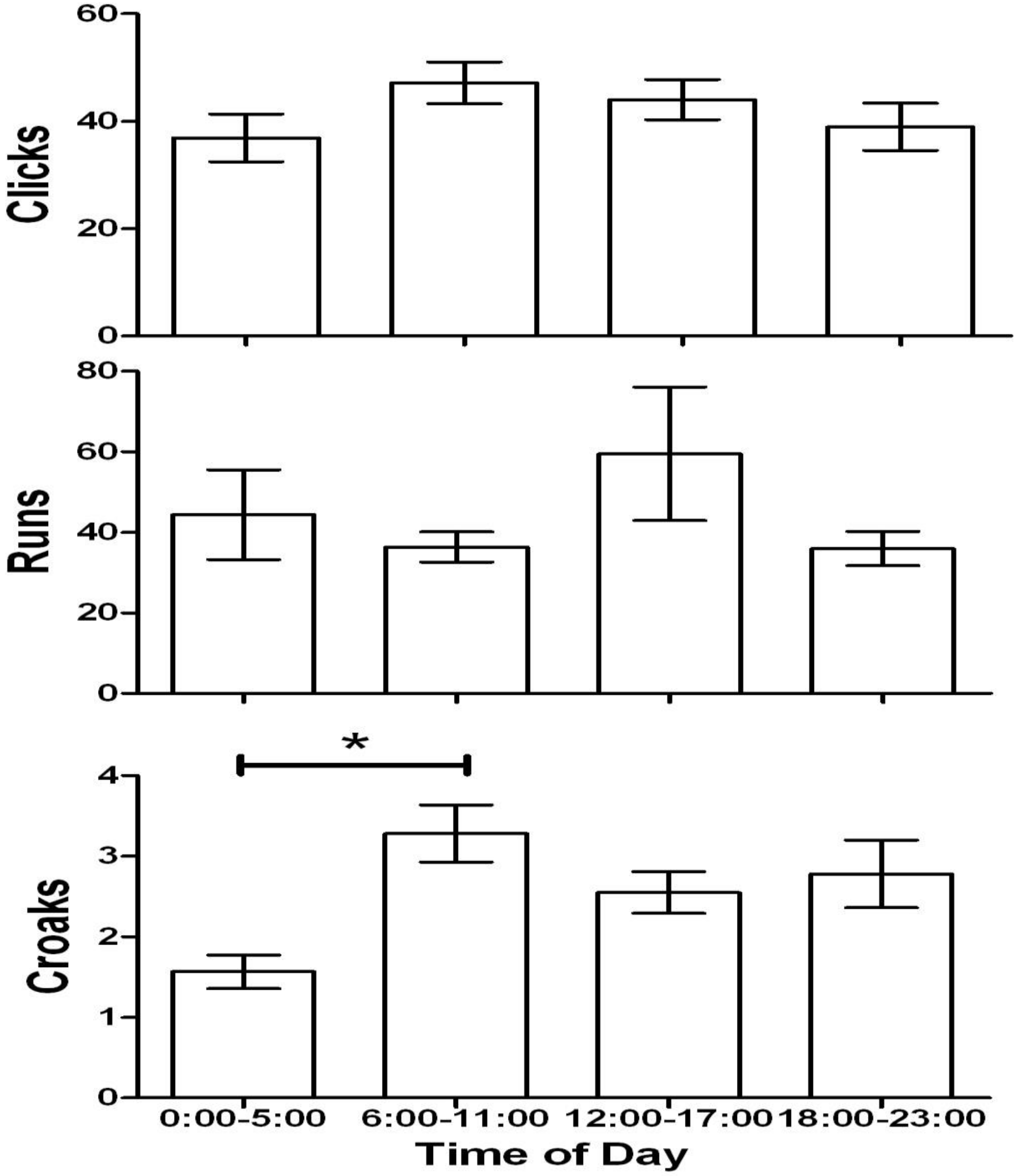

Figure 30. Mean ± SE hourly occurrences of clicks, runs, and croaks. * denotes significant difference (ANOVA).

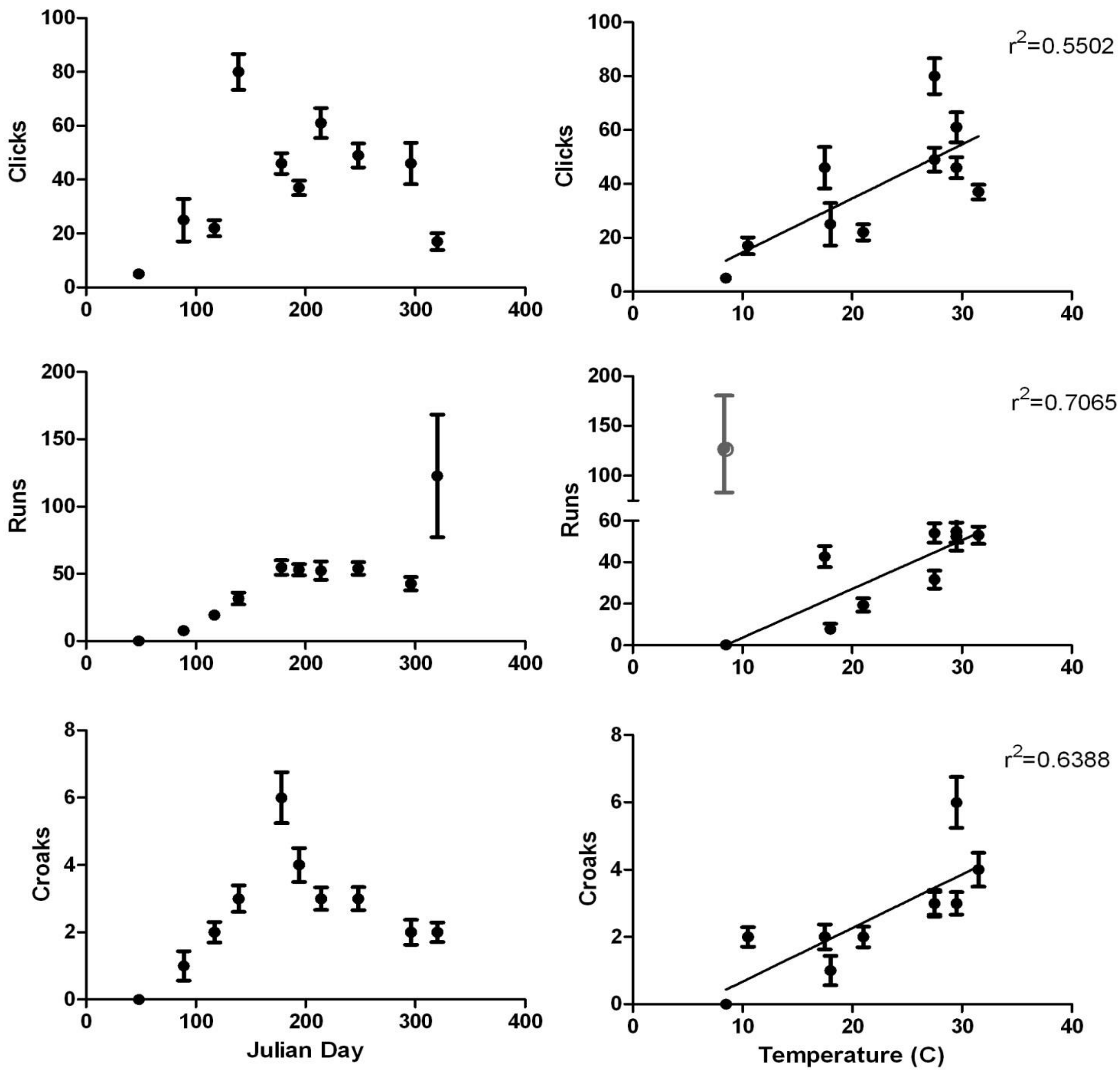

Figure 31. Mean ± SE occurrences of clicks, runs, and croaks versus temperature and Julian Day. Linear regression (r^2 values) are marked for occurrences versus temperature. November had an unusually high incidence of runs, and was removed from analysis but still graphed (shown in gray).

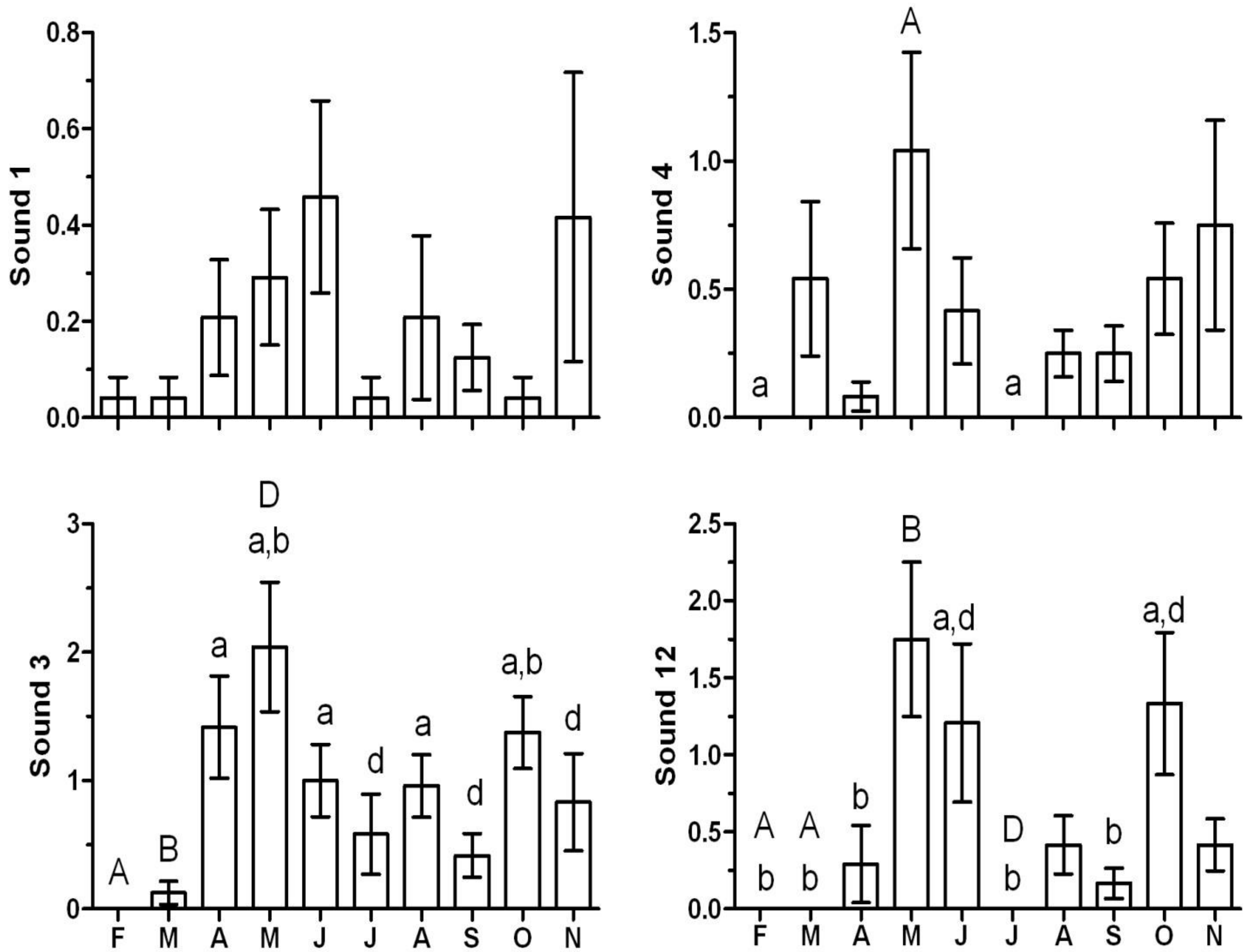

Figure 32. Mean ± SE monthly occurrences of less common sounds: 1, 3, 4, 12 Lower case letters are significantly different from uppercase letters but not significant from each other (ANOVA).

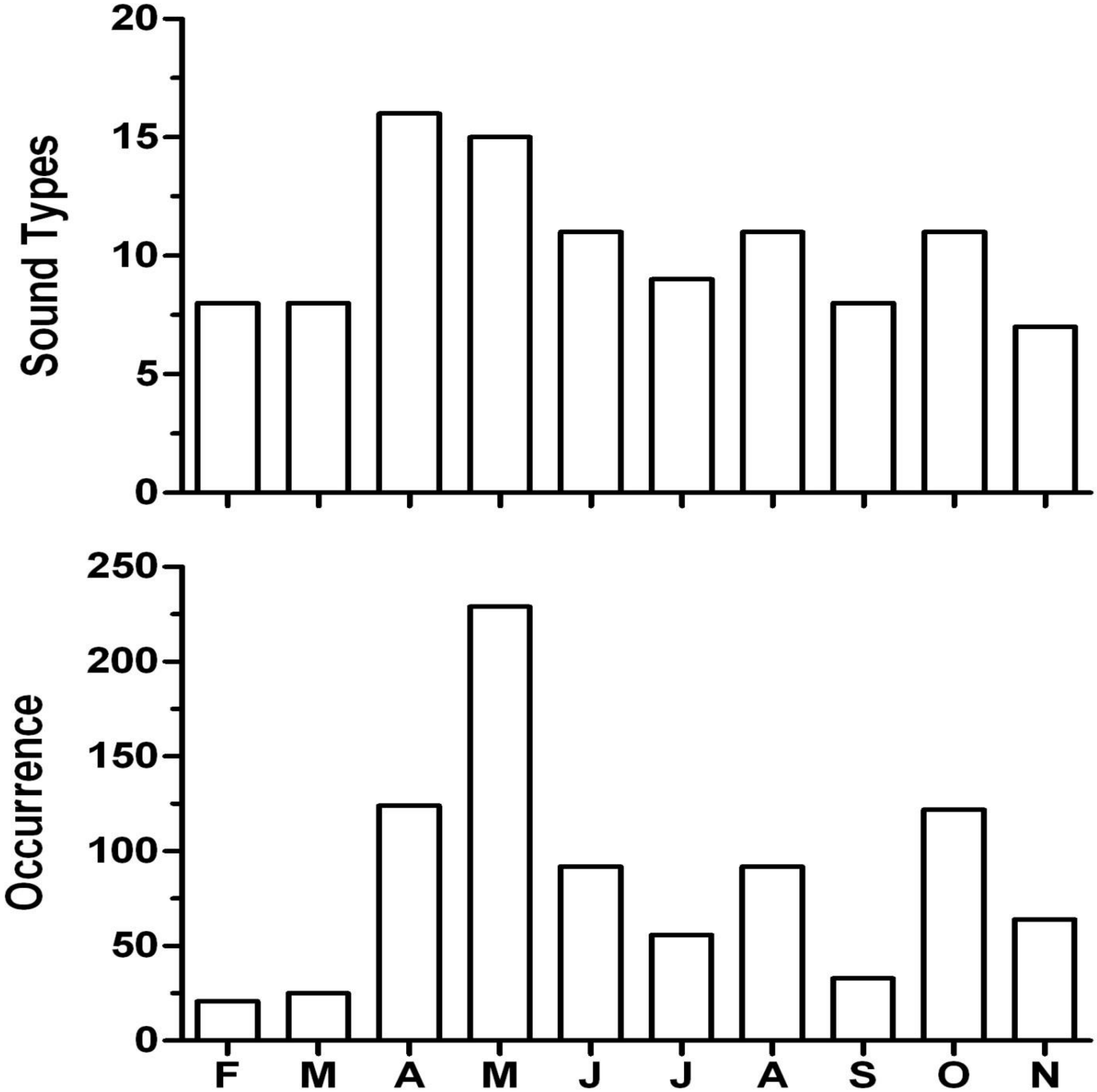

Figure 33. Total number of sound types and occurrences of sounds per month of less occurring sounds in the tidal freshwater James River. Note this does not include the 3 main sounds: runs, clicks, croaks. Total occurrence is a running tally of all sounds, excluding the 3 main sounds and sounds that only occurred during one month.

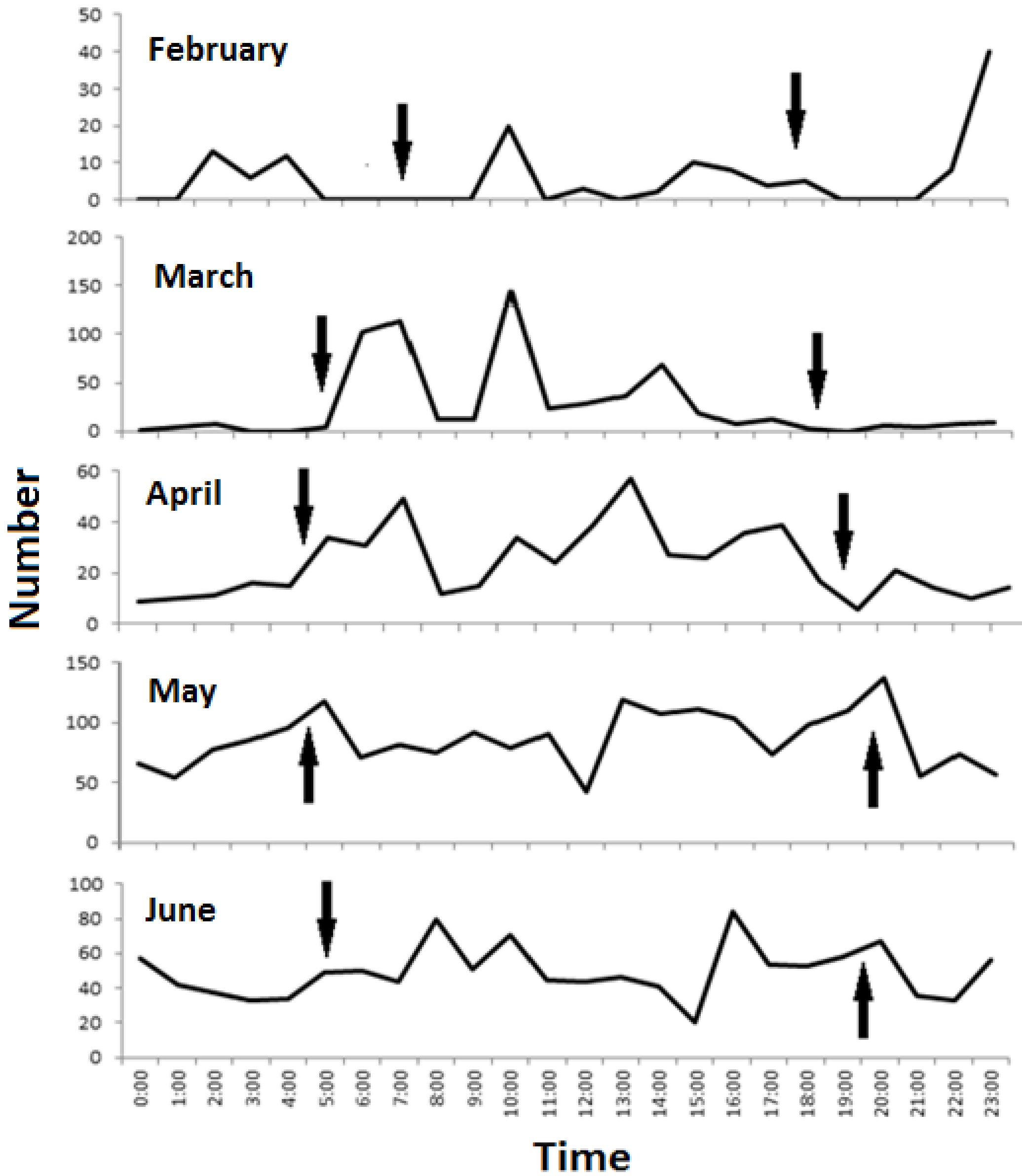

Figure 34. Daily record of clicks per 3 min interval recorded over 24 hrs monthly from February to November 2012. Arrows indicate sunrise and sunset.

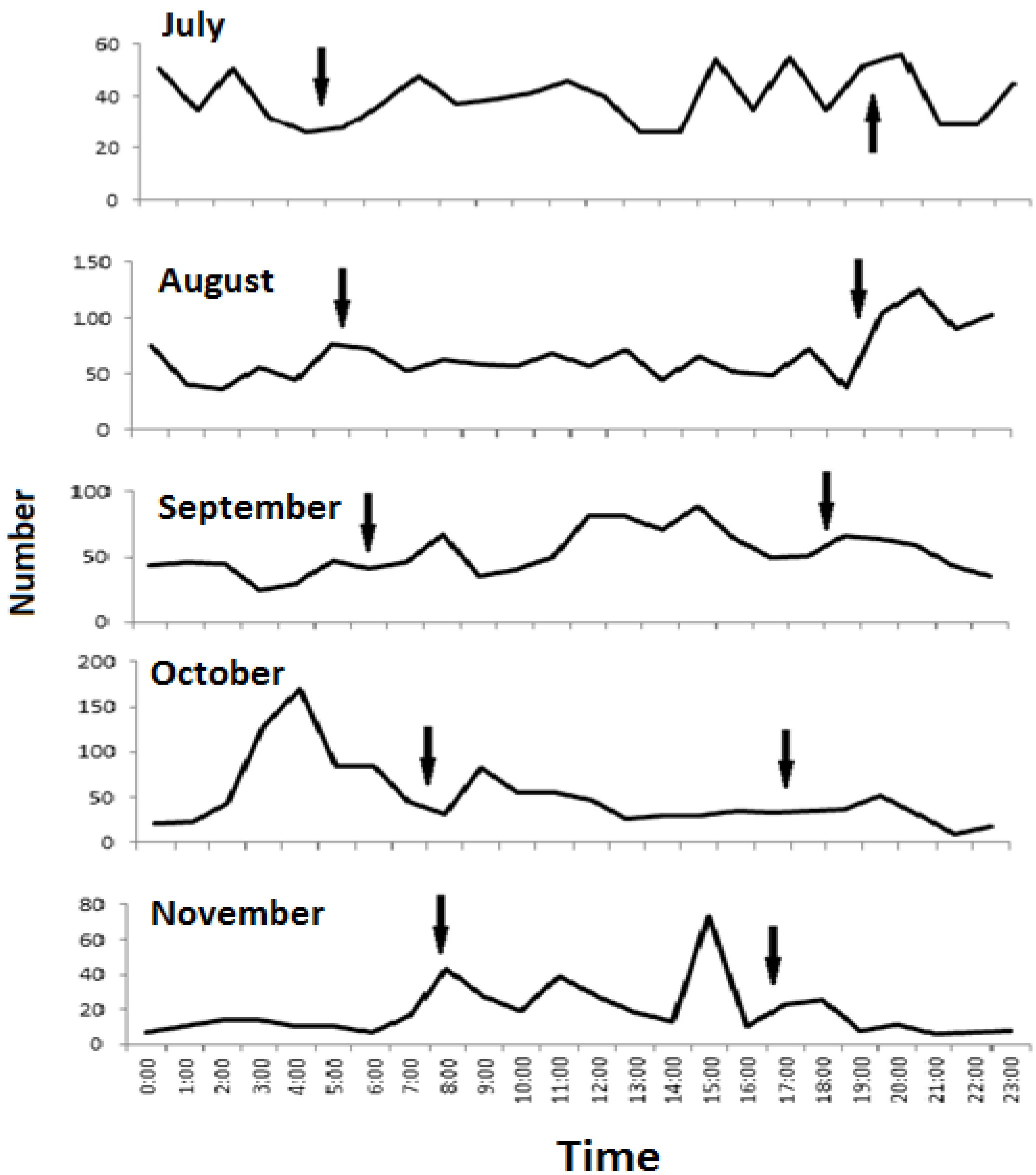

Figure 34. (continued)

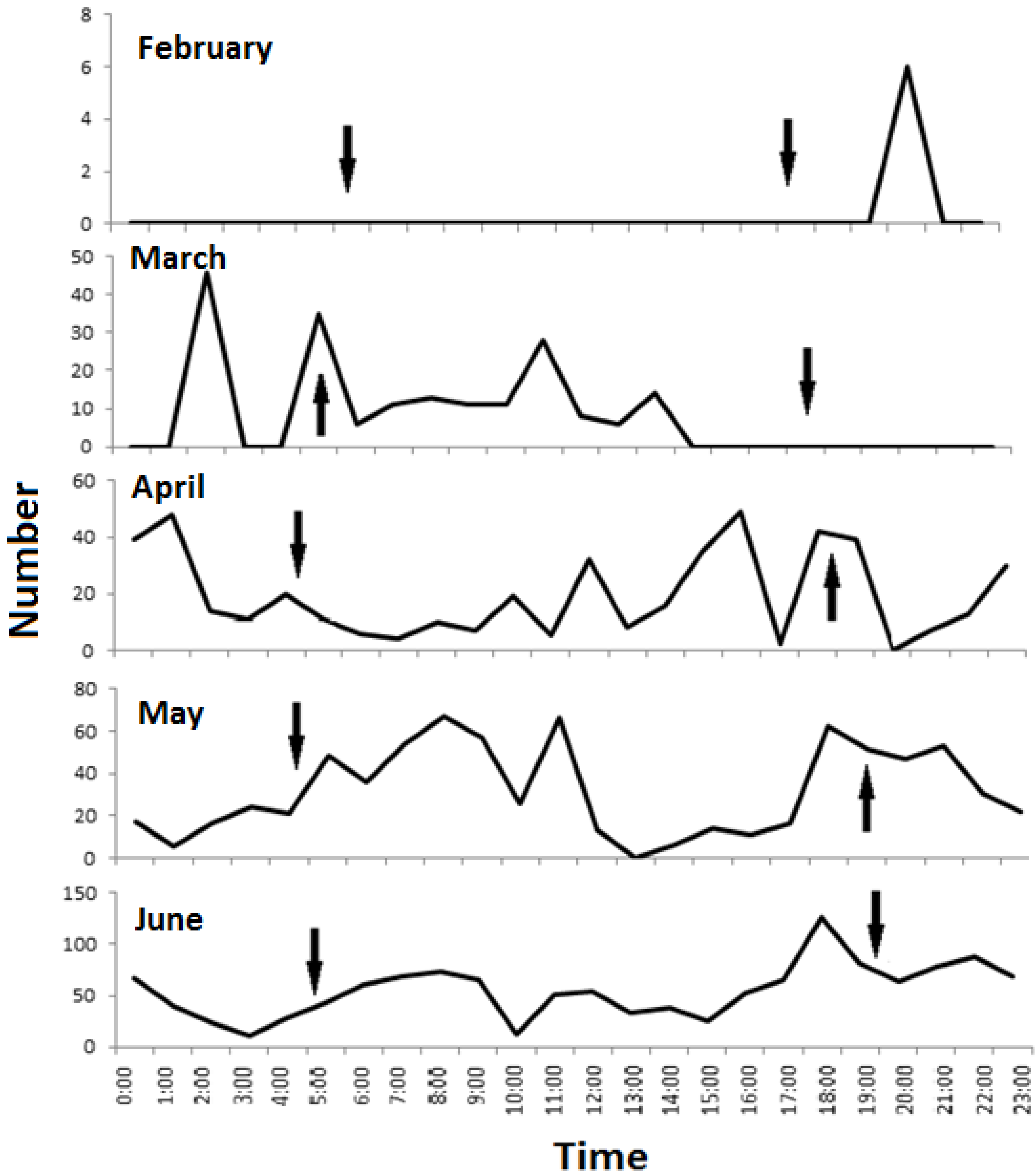

Figure 35. Daily record of runs per 3 min interval recorded over 24 hrs monthly from February to November 2012. Arrows indicate sunrise and sunset.

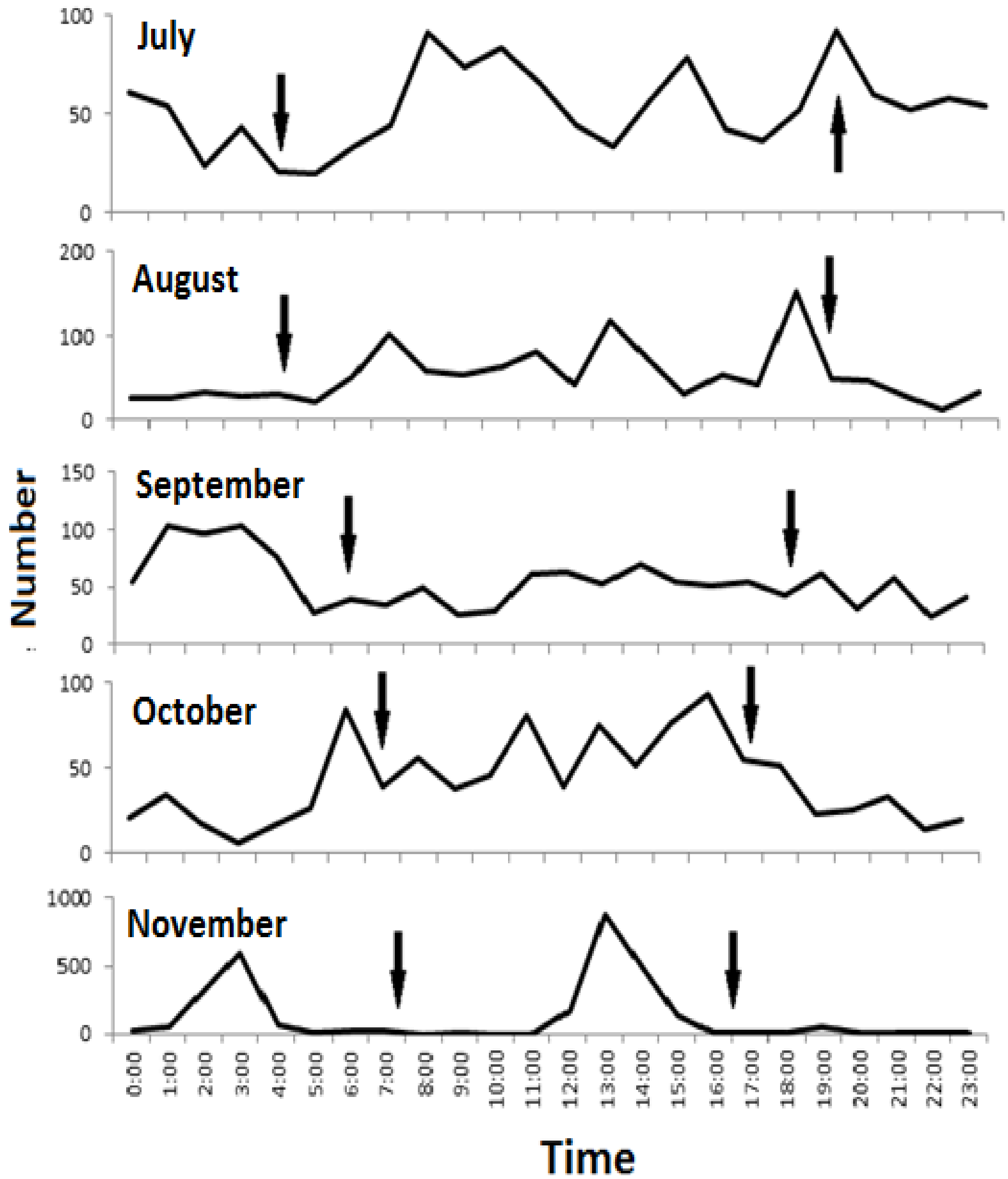

Figure 35. (continued)

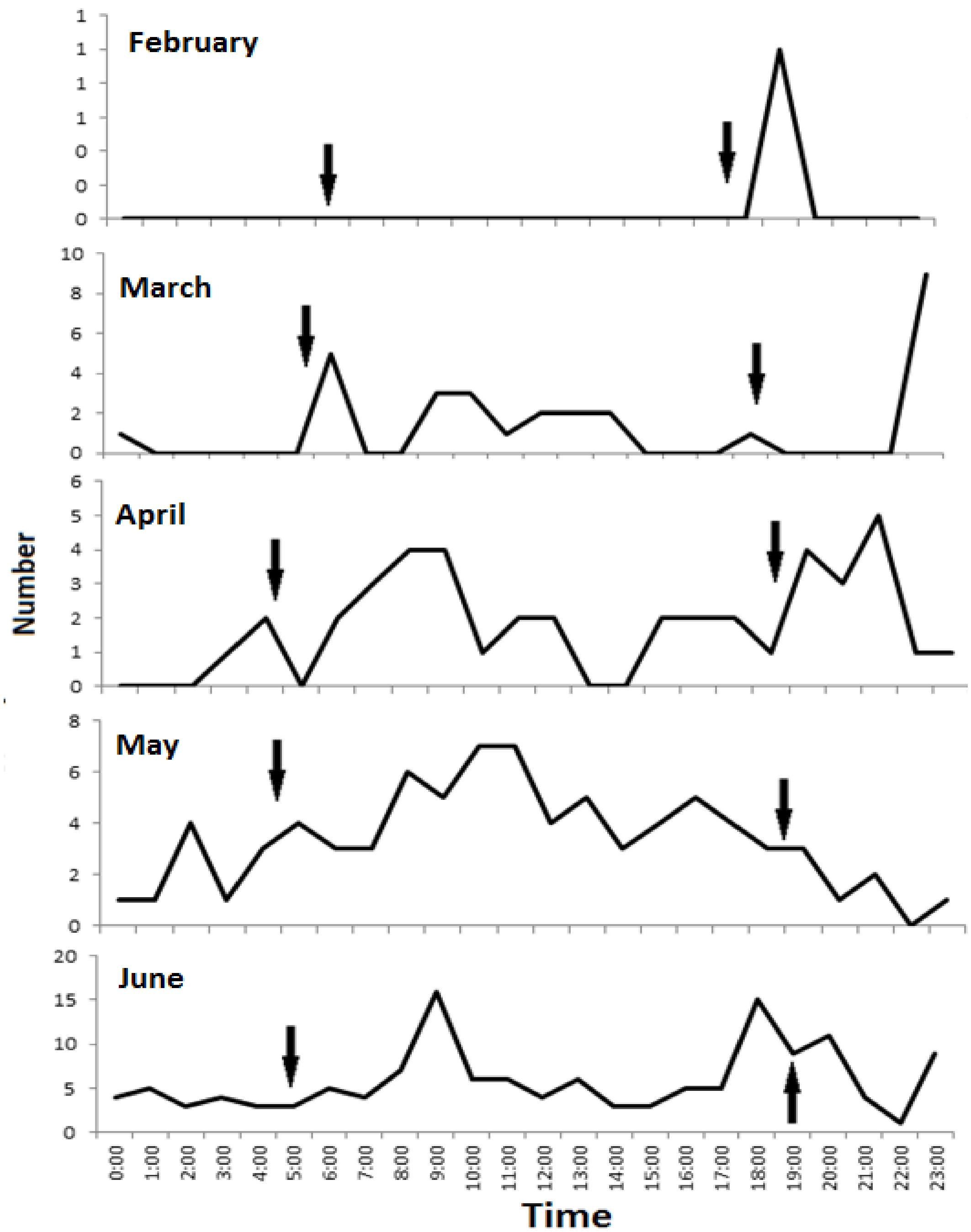

Figure 36. Daily record of croaks per 3 min interval recorded over 24 hrs monthly from February to November 2012. Arrows indicate sunrise and sunset.

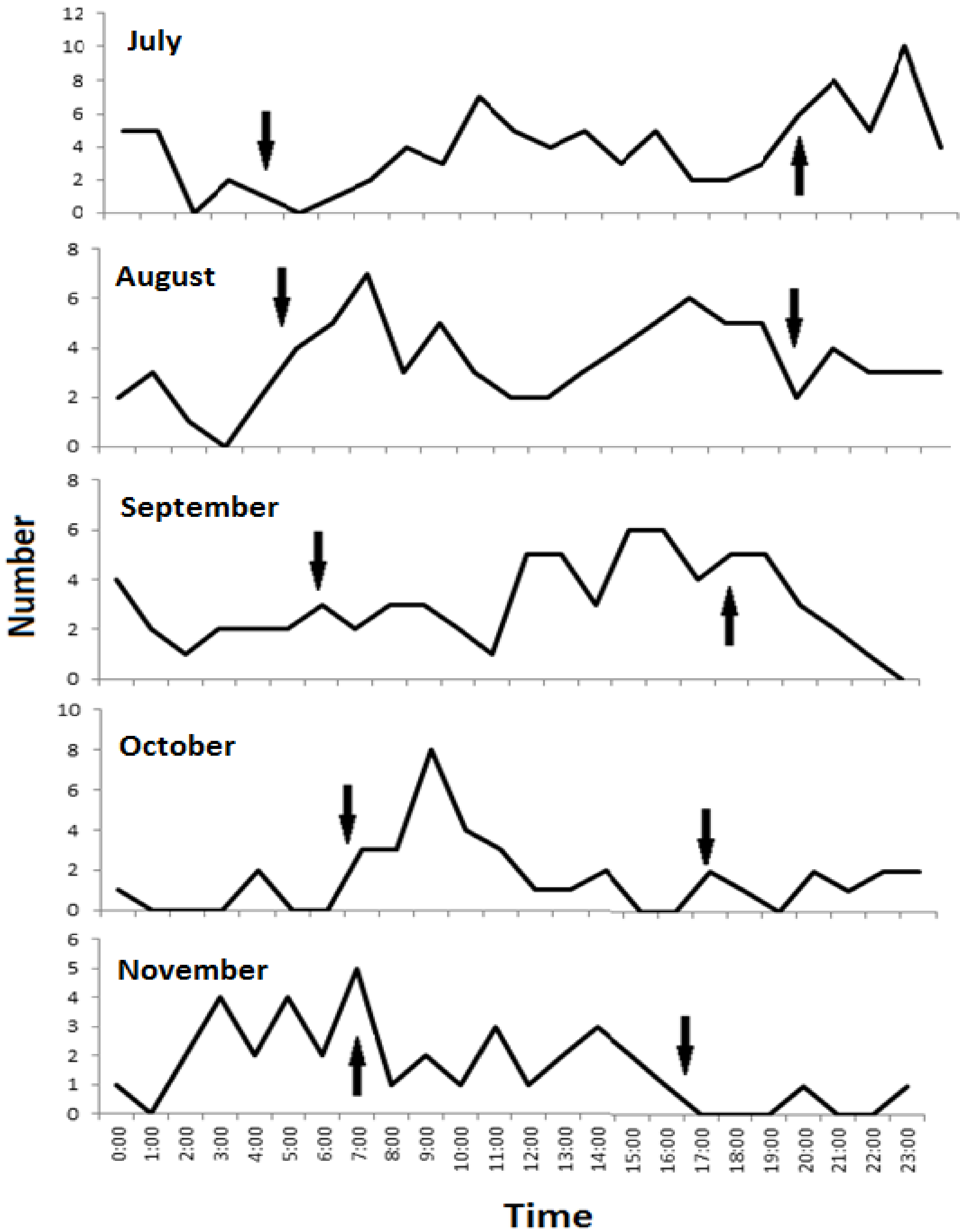

Figure 36. (continued)

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Chapter 2:

AN EXPERIMENTAL STUDY OF AGONISTIC BEHAVIOR IN JUVENILE BLUE CATFISH, *ICTALURUS FURCATUS*

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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Abstract

AN EXPERIMENTAL STUDY OF AGONISTIC BEHAVIOR IN JUVENILE BLUE CATFISH, *ICTALURUS FURCATUS*

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Blue catfish, *Ictalurus furcatus*, an invasive species in the James River, VA, are of concern due to their explosive population growth, negative impact on native species, and ability to tolerate moderate salinities to move into neighboring tributaries. I examined agonistic behavior in juveniles by introducing an intruder into a resident's territory. Fish in two size ranges (43-50 cm TL and 36-41 cm TL) were paired within the same size and across size ranges to determine size and residency impacts on territory defense. Territory was considered established when a fish entered the shelter and remained there for over an hr. Territory extablishment averaged 101 min. In 80% of trials fish entered the shelter head first and maintained that orientation. Residents lost twice, tied 7 times, and won 6 times and larger fish won 5 and lost 3 times, tying 7 times. Fish used a variety of agonistic behaviors in shelter defense including aggressive contact, caudal sweeping, and standoff behaviors. Different individuals often utilized different behaviors indicating less stereotypy than in many other fishes. Small fish performed low level aggressive behaviors more often (overswim, tunnel, rostral brush), and big fish performed higher level ones more

(caudal push). Intruders took longer to orient and approach the shelter and small fish remained adjacent to the shelter longer. Stridulation sounds were not present in territorial contests supporting the hypothesis of sound use as an anti-predator adaptation through use as a distress or alarm call.

Introduction

The Blue catfish, *Ictalurus furcatus*, an invasive species in the James River and other Chesapeake Bay tributaries, is found within 29 states through migration or introduction (Graham and DeiSanti 1999). They compromise approximately 75% of the fish biomass in the James River (Schloesser et al. 2011) and pose a health concern to residents, as their tissues exhibit high concentrations of polychlorinated biphenyls and tributyl tin (Harris and Jones 2008; Weintraub 2008). Their numbers correlate with decreasing native white Catfish populations and declines in shad spawning migrations (Schloesser et al. 2011). A better understanding of the Blue catfish will aid in management techniques that may help both native wildlife and Virginia residents.

Catfish are the highest grossing fishery in the U.S. (USDA, 2005), and *Ictalurus punctatus*, the Channel catfish, is the species often farmed. Blue catfish, although obtaining sizes up to 165 cm TL, are unpopular due to slow maturation, poor conversion of food, and low spawning rates in captivity (Graham, 1999). We have observed that these fish can become highly aggressive and can inflict spine damage when housed together. When Blue catfish are hybridized with Channel catfish, offspring have greater dress out and fillet percentages (Argue, Liu, Dunham 2003). Understanding their behavior could be important in the farming of this and other species of catfish.

With over 3,000 species, catfishes include about one-third of all freshwater fishes making them among the most successful groups of fishes (Teugels, 2003). They have highly developed chemical and auditory (Caprio and Finger 2003; Ladich and Bass 2003) but not vision (Collin 2003). Their pectoral spines are both an antipredator adaptation (Fine and Ladich, 2003; Fine et al., 1997) and capable of producing stridulation sounds (Fine et al., 1997; Kaatz et al., 2010;

Ladich and Myrberg, 2006). The Fine lab has worked extensively on the pectoral spine as an anti-predator adaptation (Bosher, Newton, and Fine 2006; Sismour et al., 2013), mechanisms of sound production (Fine et al., 1996; Fine et al., 1997; Fine et al., 2011b; Ghahramani, 2010), and the effects of predators on growth, feeding, and movement of Channel catfish (Fine et al., 2011a). We have evoked sounds in channel and Blue catfish by holding them but have not investigated the incidence of sounds in nature or the function of these sounds. Since nearly 100% of Blue catfish will produce sounds when held (Ghahramani, 2010), it is likely that acoustic communication is important in this species.

Striulation sounds could serve as an alarm call for consepecifics, increasing reproductive fitness if nearby individuals are related (Ladich and Myrberg 2006). However stridulation in Channel catfish did not signal a warning signal to largemouth bass and occurred only when a Catfish was tail first in a bass's mouth (Bosher, Newton, and Fine 2006). Distress calls could however function to attract more predators into the area and provide a chance for the fish to escape with predator aggression against one another (Matthis et al. 1995). Stridulation sounds may be used in courtship and agonistic displays as in the case of male *Corydoras* Catfishes which produce sounds more often when they are reproductively active (Pruzinszky and Ladich 1998). Competition for mates or territory evokes higher levels of aggression than competition for food (Ladich and Myberg 2006). Surprisingly there is only a single study on naturally-occurring sounds of a North American Catfish, the brown bullhead, which demonstrated that sound plays a role in submissive behavior (Rigley and Muir, 1979).

Since little is known about behavior in this invasive species, this study will examine agonistic behavior of territorial individuals when an intruder is introduced and possible stridulatory sound production occurring in contests. Due to the observation of larger Blue catfish being thrown out of tanks and cut with the pectoral and dorsal spines from other fish, I predict that larger fish will show higher levels of aggression than smaller fish. I also hypothesize that residents will win more territory contests than intruders.

Materials and Methods

Unsexed juvenile Blue catfish were collected by hook and line, electroshocking, and gill nets from the tidal freshwater James River. They were treated with 10 mg/L $KMnO_4$ for 10 min. to rid the fish of external parasites, weighed, and measured (total length:TL) ((Table 2). The largest fish were unsexed juveniles. Fish were housed in aerated 568 L black isolation tanks lined with a 2 cm layer of light-colored stones. Catfish were fed shrimp 2-3 times a week, and tanks were cleaned biweekly. Preliminary experiments were run in outdoor tanks under ambient temperature and photoperiod. Although raccoons frequently removed or injured the fish, preliminary trials allowed observations, and agonistic behavior was similar to behaviors later found in indoor trials.

Fish were kept in isolation tanks until tested. Preliminary testing showed that fish established a territory, indicated by examination and occupation of a 20L bucket shelter, usually within two hrs. The test tank of 1136 L (137x92x89 cm) with the shelter at one end was filled with water to the top of the shelter, roughly 38 cm deep. The territorial fish, defined as the original occupant of the tank, was placed in the tank for at least two hrs to establish a territory. Video recordings were made during tank explorations and territory establishment (defined as entering and remaining in the shelter for over an hr). After establishment of territory an intruder fish was introduced, and the two fish were recorded for approximately 90 min. The winner was determined by which fish was in or near the lip of the bucket at the end of the trial. All trials were conducted between 8 AM and 6 PM; previous studies showed no noticeable difference in behavior during the day.

I attempted to setup territories with cinderblock and plexiglass covers to observe behavior within the shelter. However fish did not utilize the transparent shelters but readily entered a 19 L gray paint bucket turned on its side, which limited our view of the fish within the shelter.

An HTI hydrophone (model HTI-94-SSQ) (sensitivity of -168.1 1dB re: 1V/uPa) was hung from a pvc and wire recording platform 1.4 m above the middle of the tank. It extended approximately 8 cm below the water surface and 23 cm from the shelter. Sounds were recorded with a Tascam DR 100 portable digital recorder. Video was recorded with a Surf Hero Pro video camera placed on the recording platform, allowing an aerial view of the tank. Recordings utilized the r5 setting with a resolution of 1080 pixels at 30 fps.

Sixteen trials were conducted utilizing eight fish. Four trials were conducted with four fish between 43-50 cm TL (Big), and four trials with four fish between 36-41 cm TL (Small). Another 8 trials were conducted with big fish paired with smaller fish to determine if there were size effects. Trials included reverse pairings, in which the originally territorial fish became the intruder. Trials lasted between 90-120 minutes (only 90 min were recorded). Reverse pairing trials were 2-3 days apart. Due to an equipment malfunction data from one trial's with two big fish were lost.

Behaviors were analyzed visually with windows media player, and sounds were analyzed with Raven Pro v1.3 software. An ethogram was created and behaviors were counted or measured for duration.

Statistics

A one-tailed Mann Whitney U test was conducted on paired comparisons, except standoffs, due to our expectation that there would be size and residency effects. Specific behaviors were not performed by all individuals, and individuals that did not perform a behavior were excluded from the analysis of that particular behavior. I ran a non-parametric 1way ANOVA (Kruskal-Wallis) with a Dunn's Multiple Comparison test to compare all behaviors against each other. Nonparametric tests were used due to the variability of behaviors in individual fish.

Results

Territory Establishment

Fish entered the shelter head first, and 12 of 15 remained in that position with their tails protruding. Two others faced outward, and one did not establish a territory (defined as remaining in bucket for longer than 1 hr).

Twelve fish averaged 21.1 ± 5.1 min (Mean ± SE) before first entering the shelter (range: 0.1-53.3 min). Three others required more than 2 hr to enter and were untimed; they established a territory within 5 hrs. Timed fish averaged 101.4 ± 14.4 min (0.43-138.2 min) to establish a territory. They went in and out before remaining in the shelter, averaging 7.8 ± 2.7 visits (1-32) lasting 3.4 ± 0.6 min (Figure 1). Duration of sequential visits did not change significantly (Kruskal Wallis, KC=14.98, p=0.4528), although visits 1-3 (4.73 ± 1.55) were longer than visits 4-14 (2.99 ± 0.52), excluding visits 10 and 12 (6.70 ± 1.93) (Figure 2). One fish visited 31 times before remaining in the bucket for an hr.

Contest Outcome

The fish in the shelter at the end of 90 min was labelled the winner. If both fish were inside or outside of the shelter it was considered a tie. There were 7 ties, 6 resident wins, and 2 intruder wins. The larger fish won 5 times and lost 3 times (Table 3).

Agonistic Displays

In this study, Blue catfish exhibit a wide array of behaviors which are separated into four groups (see Ethogram, Table 1). These behaviors were compared to behaviors during territory establishment and found to not be performed except when another fish was present. Shelter behaviors consist of the following: orient and approach (orienting and swimming toward the shelter), entering and leaving shelter, entrance baulk (swimming toward shelter and backing up or turning around without entering), shelter turn around (turning around inside shelter), shelter adjacent (remaining still on the side of the shelter), and shelter standoff (fish remains still facing opening with fish inside).

In sweeping behaviors both fish are in close proximity (within 31 cm) and the caudal fin moves slowly back and forth with or without contacting the opponent. This behavior can be performed by one or both fish simultaneously. In mutual sweeping fish are either parallel or antiparallel.

Aggressive behaviors were categorized in ascending level of aggressiveness based on ability to displace fish and included: brushing the pectoral fin or rostrum across the side of the opponent, standoff with or without contact (fish remains still while facing or touching opponent), pivoting (turning along sagittal axis in a herding fashion), resting head on opponents head, swimming above (overswim) or under (tunneling) the opponent, pushing with either the rostrum or caudal region and displacing opponent, and lateral head contact with pivot (LHCP: pushing the opponent with the side of head while pivoting along the sagittal axis).

Other behaviors include: bumping into the opponent, pectoral jerking (quickly jerking one or both pectoral fins forward in a jagged motion-not stridulation), pectoral fanning in place or while backing up, and releasing a bubble from the gills.

More fish performed caudal fin sweeps with and without contact, caudal push, and shelter turn around than shelter behaviors such as: entrance baulk, partial enter, and back up (Figure 3). Pectoral brush and jerk were not common. Caudal fin sweeps with (132.9 ± 40.4) and without contact (82.5 ± 11.9), as well as mutual caudal fin sweep with (85.9 ± 20.5) and without contact

(82.8 ± 13.4) occurred most commonly. Lateral head contact with pivot also had significantly more occurrences (77.8 ± 21.8) than behaviors such as pectoral brush (4.1 ± 1.2), bubble (1.8 ± 0.3), shelter protrusion (3.1 ± 0.6), and leaving the shelter (4.9 ±0.8) (KS=210.5, $p<0.0001$) (Figure 4).

Typical Encounters

When a first introduced, an intruder typically orients and approaches the shelter. This is followed by either an entrance baulk, partial, or full entrance. Occasionally a fish will remain on the opposite side of the tank from the shelter or remain adjacent to the shelter for an extended period. Upon entering the shelter the intruder usually has a bump followed by a rostral or pectoral brush. The resident often turns around inside of the shelter or begins caudal fin sweeping with or without contact. If the intruder does not immediately leave caudal and rostral pushes, lateral head contact with pivots, rostral or pectoral brushes, and overswim or tunnel are likely to occur. Caudal fin sweeps usually precede an aggressive encounter but they also occur together. If neither fish conceded, a standoff with or without contact began. A fish leaving the shelter may be pursued by the other fish often with pivoting and aggressive or sweeping behaviors. Shelter orientation and lineup follow, and one or both fish would re-enter the shelter and continue aggressive displays and standoffs.

Size affected both aggressive and shelter behaviors: small fish tended to perform low level aggressive behaviors (rostral brush, overswim, and underswim) and big fish higher level ones (pivot and caudal push) (Figure10). Small fish remained adjacent to the side of the shelter longer (3345 ± 862.9 sec) than big fish (324.0 ± 251.1 sec) (U=2.000, p=0.0242) (Figure 12). They brushed their rostrum against opponent (29.9 ± 14.9) more often than big fish (3.3 ± 0.8)

(U=14.50, p=0.0111). They were also more likely (U=15.00, p=0.0226) to overswim (12.2 ± 3.4 vs 3.5 ±1.3) and tunnel (3.5 ±0.8 vs 1.3 ±0.2) (U=5.00, p=0.0183) than big fish. Big fish (14.7 ± 3.1) pushed with their caudal region more than small fish (8.6 ±1.2) (U=27.00, p=0.0472).

Big fish were also more likely to break a standoff with an aggressive behavior (13.5 ± 2.9) than small fish (6.8 ± 1.1) (U=16.00, p=0.0301). Fish, regardless of size or residency break a standoff more often with aggressive behaviors (17 fish, 9.9 ± 1.7 times) than escaping (5 fish, 1.4 ± 0.2 times) (U=5.500, p=0.0020) (Figure 11). Standoffs with contact had a bimodal distribution with fish often antiparallel (180 degrees) or parallel (0 degrees), with a few others between 10-60 degrees (Figure 5 B).

Residency status affected behaviors measured in duration. Intruders took (U=3.500, p=0.0025) longer (61.7 ± 25.6 sec) to orient toward the shelter and approach it than residents (9.2 ± 2.7 sec). They also rested their head longer on opponents (57.7 ± 22.2 sec) than residents (7.0 ± 1.0 sec) (U=0.000, p=0.0383), although only 6 fish exhibited this behavior.

Small fish performed aggressive behaviors more often and with more individuals (1-3 vs. 2-8) when paired with big than with small fish. A Mann-Whitney U test could not be run on many behaviors due to the occurrence of behavior in two or less fish for similar pairings (B-B, S-S). Big fish also exhibited more behaviors with more individuals (2-5 vs. 1-8) with unlike pairings (B-S, S-B) (Figure 13).

Stridulation sounds were not found in any of the trials.

Discussion

There has been little work on the behavior of North American Catfish or naturally occurring sounds, and this study is the first to examine agonistic behavior in Blue catfish, one of the largest fishes in North America. Blue catfish demonstrate a wide array of behaviors and individual fish vary in their utilization of these behaviors. Many other fishes have fewer and more stereotyped behaviors, such as lateral displays, headbutts, and chasing (Kramer and Bauer, 1976; Keenleyside and Yamamoto, 1962). Standoffs share some similarity with lateral displays, however we could not observe dorsal fin extension due to overhead recording. A study in juvenile African Catfish, *Clarias gariepinous*, noted only biting as an aggressive display (Kaiser, Weyl, and Hecht 1995), a behavior also seen in other fishes (Peak, Matos, and Mcgregor, 2006). This study documents a number of behaviors not described in catfishes.

Surprisingly, a number of contests resulted in ties with no determination of dominance, although residents won more than they lost. Bigger fish also won contests more often, but they did not win invariably. Size and age can increase the chances of winning contests, with even small differences in length, less than 0.1 mm, having an impact in some species of fish (Alcazar et al. 2014). Although there was only a 2 cm difference between the biggest small fish and the smallest big fish, there was a 180 g mass difference between them (Table 2). Low level aggressive displays were more likely to be performed by small fish, and big fish tended to perform higher level aggressive displays that involved displacing their opponent. A possible exception to this increased level of aggression in larger fish is lateral head contact with pivot (LHCP), which occurred 51 times more often when small fish were paired with big ones, although it did not reach significance.

Based on preliminary observations adult Blue catfish are more aggressive than juveniles. In preliminary observation Blue catfish, greater than 65 cm TL, were observed tunneling under an opponent to the point of pushing it out of the water. Larger fish with cuts on their lateral and ventral surfaces were found on the floor outside of tanks covered with plastic screens held down by bricks. By contrast small fish, approximately 30 cm TL, tended to remain in close proximity in a community tank. More aggression in bigger individuals in this study suggests a trend toward the adult pattern of aggressiveness.

Because Blue catfish are not known for their visual abilities (Collins 2003) they may rely more heavily on chemical and auditory cues (Caprio and Finger 2003; Ladich and Bass 2003). Many of the behaviors they performed involved touching the opponent in some way, it is also likely that Blue catfish rely on the sensation of touch in communication. Barbels were seen to twitch when they came into contact with the bucket or side of the tank, although they did not appear to be used in any display.

Caudal fin sweeps with and without contact were the most common among fish and appeared to be a low level aggressive display that stimulated the opponent into performing more aggressive displays. Lateral head contact with pivot was the second most used display and one of the more forceful ones (equal to rostral and caudal push).

The dorsal and pectoral spines may be used in agonistic displays with threats through tunneling or pectoral brushing. Although juvenile Blue catfish were not injured by the spines, I have seen larger adults cut down to the muscle laterally and ventrally. In the transport of specimens some died from injuries sustained from other fish’s spines to the skull or side suggesting high levels of aggression. Smaller fish were observed to have thinner but sharper spines.

The individual variation among the fish may be due to multiple factors that could be lessened with a larger sample size. Familiarity with shelter and other fish could have played a role (Slavik, Maciak, and Horky 2012; Dijkstra et al. 2008) especially in the winners of contests in the reverse pairings. It is likely that these fish had come into contact with other blue catfish within the James River prior to being caught. Also the specimens, due to their similar size ranges, could be from the same clutch or cohort. Individual personality can play a role in the type and number of agonistic displays in the individual and opponents (Hamilton and Ligocki 2012; Matessi et al. 2010). This may account for the observation that both big and small fish performed aggressive behaviors more often and in more fish when paired with different sized fish. Factors such as temperature and food availability can increase aggressiveness (Toobaie and Grant 2013).

Due to the short time given to establish a territory (2-5 hrs), fish may have more readily share the territory with an intruder due to lack of time investment. This could explain the outcome of a tie even with ongoing aggression during the contest. Blue catfish would not utilize a shelter with a clear top, indicating that the absence of light is what they prefer when choosing a shelter. In this sense, the shelter could have been perceived as a hiding place rather than a nest, although a limited resource such as this could still be worth defending in the wild.

Nearly 100% of Blue catfish will make stridulatory sounds when held (Ghahramani, 2010) however juvenile Catfish did stridulate when defending a shelter. Younger fish were used due to the size limitations of my tanks and availability of specimens. Therefore I cannot rule out that adults use sounds in agonistic and courtship displays, such as in *Corydoras* Catfish (Pruzinszky and Ladich 1998). However, recordings in the tidal freshwater James River during the spawning season did not include Catfish stridulation, and this casts doubt on this possibility. Observations thus far indicate that Blue catfish are only known to produce sounds when held. Channel catfish stridulated when held tail-first in the mouth of a largemouth bass (Bosher, Newton, Fine 2006). It is therefore possible that stridulation sound functions as a distress call.

Table 1. Blue catfish ethogram.

ID	Behavior Code	Description
Shelter Behaviors		
ID	**Behavior Code**	**Description**
SA	Shelter Adjacent	Fish remains still on the side of the shelter.
SOA	Shelter Orient and Approach	Fish orients to shelter and swims toward opening.
EB	Entrance Baulk	Fish swims towards opening of shelter and then backs up or turns around. Usually preceded by shelter line up.
PSE	Partial Shelter Enter	Fish partially enters shelter and leaves before fully entering.
SE	Shelter Enter	Fish enters shelter (recorded when the rostrum crosses the lip). The fish can enter with or without opponent fish inside the shelter.
SBU	Shelter Back Up	Fish backs out of the shelter
STA	Shelter Turn Around	Fish turns around inside the shelter to face the other direction. Can be done by swimming forward or backwards and can be followed by either a partial or full leave of the bucket.
SP	Shelter Protrusion	Fish protrudes head from the bucket without leaving.
LS	Leaves Shelter	Fish exits shelter, recorded when the tip of the caudal fin crosses the lip of the shelter.
STOs	Shelter Standoff	Fish remains still while facing shelter opening and opponent inside.
Sweeping Behaviors		
ID	**Behavior Code**	**Description**
SCFS	Shelter Caudal Fin Sweep	Fish sweeps its caudal fin in a short back and forth motion across the opening of the shelter while slowly pivoting along sagittal axis.
CFS	Caudal Fin Sweep	Fish sweeps its caudal fin back and forth, without contacting nearby opponent. Not used for propulsion.
CFSw/c	Caudal Fin Sweep with contact	Fish sweeps its caudal fin back and forth across part of opponent’s body. Not used for propulsion.
CFSm	Mutual Caudal Fin Sweep	Fish are usually parallel (can be antiparallel) and sweep their fins back and forth without contact. Roughly equal number of sweeps per fish.
CFSmw/c	Mutual Caudal Fin Sweep with contact	Fish are usually parallel (can be antiparallel) and sweep their fins back and forth with contact. Roughly equal number of sweeps per fish.

Table 1: (continued).

Aggressive Behaviors (in order of least to most aggressive based on amount of displacement)		
ID	**Behavior Code**	**Description**
RBRSH	Rostral Brush	Fish brushes rostrum along the side of opponent either parallel or antiparallel.
PBRSH	Pectoral Brush	Fish brushes pectoral fin along the side of opponent either parallel or antiparallel.
STO	Standoff	Fish remains still within 30 cm of opponent. Rostrum points towards a body part of opponent.
STOw/c	Standoff with contact	Fish remains still while in contact with opponent
PVT	Pivot	Fish pivots back and forth along it sagittal axis, turning its head towards opponent in a herding fashion without contact.
HR	Headrest	Fish positions head on top of another fish's head for more than 5 seconds
TN	Tunneling	Fish swims underneath of opponent
OS	Overswimming	Fish swims overtop of opponent
RP	Rostral Push	Fish pushes rostrum into another fish causing a small displacement of the other fish
CP	Caudal Push	Fish either parallel or anti parallel to opponent and pushes against it with mid part of caudal fin, causing a small displacement of opponent.
LHCP	Lateral Head Contact with Pivot	Fish pivots along sagittal axis, bumping the side of its head against opponent, often causing a small displacement of the other fish.

Table 1: (continued).

Other Behaviors (unknown to be submissive or aggressive)		
ID	**Behavior Code**	**Description**
B	Bump	Fish bumps into opponent with its rostrum after initial exploration of tank.
PJ	Pectoral Jerk	Fish swiftly jerks one or both pectoral fins forward in a jagged motion.
PF	Pectoral Fanning	Fish fans pectoral fins without contact or forward motion.
BPF	Backwards Movement with Pectoral Fanning	Fish pivots back and forth while swimming backwards away from opponent while fanning pectoral fins.
BBL	Bubble	Fish releases bubbles from its gills
BRAa	Breakaway-aggressive	First fish to leave a standoff either by swimming forward or pivoting head away followed by aggressive behaviors
BRAe	Breakaway-escape	First fish to leave a standoff either by swimming forward or pivoting head away followed by swimming away from opponent.

Table 2: Weights and total lengths (TL) of Blue catfish used in this study.

Fish ID	***Weight (g)***	***TL (cm)***
B1	1120	50
B2	1081	50
B3	726	43
B4	741	45
S1	546	41
S2	461	38
S3	523	39
S4	466	36

Table 3: Encounters of resident and intruder Blue catfish. Big fish (B1-B4) and small fish (S1-S4). Residents who won are marked with *, and larger fish who won are marked with ^.

Resident	*Intruder*	*Winner*
B1	B2	B1 *^
B4	B3	Tie
B2	B1	Tie
S1	S3	S1 *^
S2	S4	S2 *
S3	S1	Tie
S4	S2	S4 *^
B2	S1	Tie
B1	S3	S3
B3	S2	Tie
B4	S4	B4 *^
S1	B2	S1 *
S3	B1	B1 *^
S4	B4	Tie
S2	B3	Tie

Table 4: Mean Occurrence ± SE (sec), sample size, and range of shelter behaviors for resident, intruder, big, and small Blue catfish including Entrance Baulk (EB), Partial Shelter Enter (PSE), Shelter Enter (SE), Shelter Backup (SBU), Shelter Turn Around (STA), Shelter Protrusion (SP), and Leave Shelter (LS)

Variable	*EB*	*PSE*	*SE*	*SBU*	*STA*	*SP*	*LS*
Resident	2.0 ± 0.0	2.0 ±0.6	4.6 ± 1.3	4.0 ± 2.4	8.8 ± 2.9	3.6 ± 1.3	4.7 ± 1.2
	1 (2)	3 (1-3)	8 (1-10)	6 (1-16)	11 (1-32)	7 (1-10)	9 (1-10)
Intruder	4.9 ± 1.2	4.3 ± 0.8	4.9 ± 1.2	11 ± 6.0	7.8 ± 2.0	2.8 ± 0.4	5.1 ± 1.2
	8 (2-12)	6 (1-6)	11 (1-12)	2 (5-17)	10 (1-21)	9 (1-4)	8 (1-11)
Big	4.8 ± 1.9	3.4 ± 0.9	1.7 ±0.5	5.6 ± 2.9	6.0 ± 2.0	2.1 ± 0.5	4.4 ± 1.3
	5 (2-12)	5 (1-6)	7 (1-4)	5 (1-17)	10 (1-21)	7 (1-4)	8 (1-11)
Small	3.0 ± 0.4	3.8 ± 1.1	3.9 ±0.9	6.0 ±5.0	10.5 ± 2.8	3.9 ± 0.9	5.4 ± 1.2
	4 (2-4)	4 (1-6)	9 (1-10)	3 (1-16	11 (1-32)	9 (1-10)	8 (2-10)

Table 5: Mean Occurrence ± SE (sec), sample size, and range of sweeping behaviors for resident, intruder, big, and small Blue catfish including Caudal Fin Sweep without (CFS) and with contact (CFSw/c), and Mutual Caudal Fin Sweep without (CFSm) and with contact (CFSmw/c)

Variable	***CFS***	***CFSw/c***	***CFSm***	***CFSmw/c***
Resident	160. 9 ± 69.1	76.1 ± 15.9	76.1 ± 15.9	83.3 ± 28.3
	11 (26-809)	12 (12-207)	12 (12-207)	8 (8-250)
Intruder	102.1 ± 39.8	90.1 ± 18.5	90.1 ± 18.5	83.3 ± 28.3
	10 (12-384)	10 (17-184)	10 (17-184)	8 (8-250)
Big	177.9 ± 68.4	74.5 ± 17.0	74.5 ± 17.00	92.9 ± 26.8
	12 (12-809)	12 (12-184)	12 (12-184)	8 (8-250)
Small	72.9 ± 12.2	92.0 ± 16.8	92.0 ± 16.8	73.6 ± 29.3
	9 (19-123)	10 (17-207)	10 (17-207)	8 (8-250)

Table 6: Mean Occurrence ± SE (sec), sample size, and range of aggressive behaviors for resident, intruder, big, and small Blue catfish including Rostral Brush (RBRSH), Pectoral Brush (PBRSH), Pivot (PVT), Tunnel (TN), Overswim (OS), Rostral Push (RP), Caudal Push (CP), and Lateral Head Contact with Pivot (LHCP)

Variable	***RBRSH***	***PBRSH***	***PVT***	***TN***	***OS***	***RP***	***CP***	***LHCP***
Resident	22.3 ± 15.1	6.8 ± 2.3	42.1 ± 15.4	3.5 ± 1.2	9.1 ± 3.7	13.6 ± 3.3	11.3 ± 1.9	81.0 ± 23.8
	9 (1-141)	4 (1-11)	9 (3-136)	4 (1-6)	8 (1-26)	8 (2-29)	10 (3-10)	9 (1-182)
Intruder	10.9 ± 5.5	2.3 ± 0.8	24.0 ± 6.3	1.9 ± 0.4	7.2 ± 2.6	19.8 ± 12.3	12.6 ± 3.4	74.9 ± 36.9
	9 (1-53)	6 (1-6)	10 (1-65)	(1-4)	9 (1-26)	9 (1-116)	10 (1-41)	10 (5-394)
Big	3.3 ± 0.8	3.8 ± 2.1	40. ± 15.3	1.3 ± 0.2	3.5 ± 1.3	11.3 ± 2.9	14.7 ± 3.1	47.0 ± 18.6
	9 (1-9)	4 (1-10)	8 (8-136)	6 (1-2)	8 (1-10)	10 (1-29)	11 (3-41)	9 (1-178)
Small	29.9 ± 14.9	4.3 ± 1.6	26.8 ± 8.6	3.5 ±0.8	12.2 ± 3.4	10.6 ± 3.1	8.6 ± 1.2	105.5 ± 36.8
	9 (1-141)	6 (1-11)	11 (1-95)	6 (1-6)	9 (1-26)	7 (2-21)	9 (1-13)	10 (5-394)

Table 7: Mean Occurrence ± SE (sec), sample size, and range of other behaviors for resident, intruder, big, and small Blue catfish including Bump (B), Pectoral Jerk (PJ), Bubble (BBL), Breakaway Aggressive (BRa), and Breakaway Escape (BRe)

Variable	***B***	***PJ***	***BBL***	***BRa***	***BRe***
Resident	9.0 ± 2.0	13.4 ±5.5	1.0 ± 0.0	9.8 ± 2.0	1.0 ± 0.0
	7 (2-16)	5 (2-32)	4 (1)	9 (2-24)	2 (1)
Intruder	8.8 ± 1.8	31.8 ± 21.1	2.1 ± 0.4	10.1 ± 2.8	1.7 ± 0.3
	12 (1-21)	5 (5-115)	8 (1-3)	8 (1-25)	3 (1-2)
Big	7.2 ± 1.5	42.7 ± 36.2	1.7 ± 0.3	13.5 ± 2.9	1.5 ± 0.5
	10 (1-16)	3 (6-115)	10 (1-3)	8 (2-25)	2 (1-2)
Small	10.7 ± 2.2	14.0 ± 4.4	2.0 ± 1.0	6.8 ± 1.1	1.3 ± 0.3
	9 (1-21)	7 (2-32)	2 (1-3)	9 (1-12)	3 (1-2)

Table 8: Mean Duration ± SE (sec), sample size, and range of behaviors for resident, intruder, big, and small Blue catfish

Variable	*SOA*	*SA*	*HR*	*PF*	*BPF*
Resident	9.2 ± 3.7	1829.0 ± 1609.0	7.0 ± 1.0	145.0 ± 46.2	229.4 ± 156.3
	5 (3-23)	3 (26-5039)	3 (5-8)	6 (11-283)	5 (11-842)
Intruder	61.7 ± 25.6	2780.0 ± 891.1	57.7 ± 22.2	120.7 ± 34. 9	59.4 ± 15.6
	13 (13-356)	8 (123-6141)	3 (33-102)	7 (22-265)	8 (8-134)
Big	73.1 ± 37.1	324.0 ± 251.1	48.3 ± 28.5	104.9 ± 40.34	52.7 ± 14.7
	9 (3-356)	3 (26-823)	3 (5-102)	7 (11-283)	6 (8-107)
Small	21.1 ± 3.4	3345 ± 862.9	16.3 ± 8.3	163.5 ± 35.38	186.6 ± 112.0
	9 (4-34)	8 (421-6141)	3 (8-33)	6 (22-265)	7 (11-842)

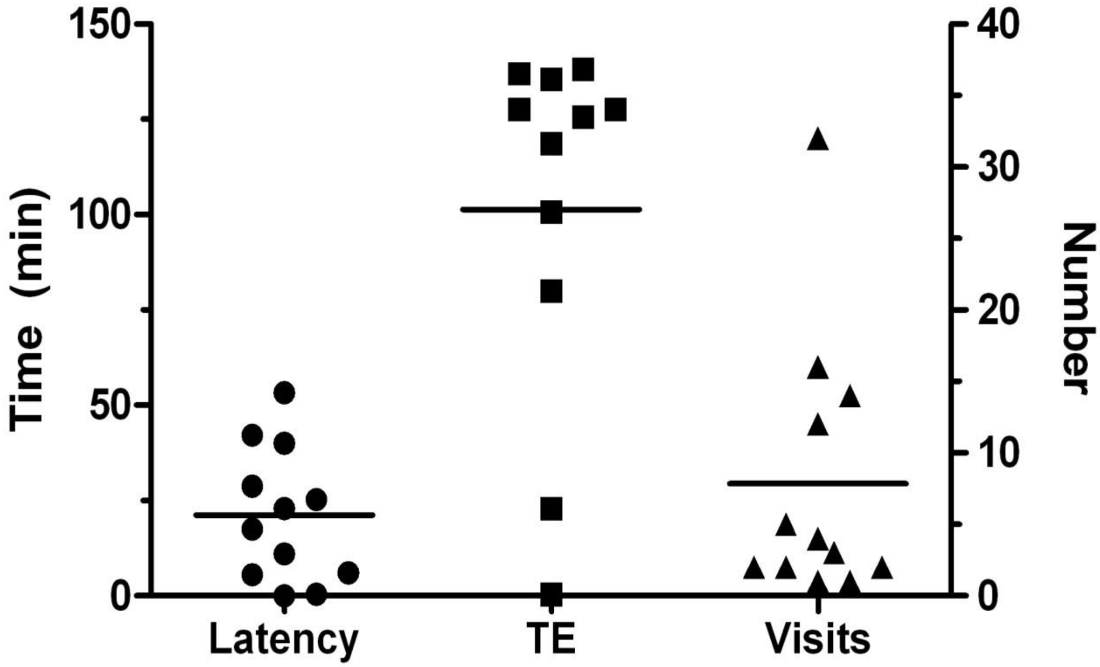

Figure 1. Mean latency to first enter shelter (min), time to establish a territory (TE) (remaining in the shelter for at least 1 hr), and number of visits before establishing a territory for juvenile Blue catfish. Three fish that required more than 2 hrs first enter or establish a territory are not included. One fish that did enter the bucket but did not establish a territory was not counted for TE.

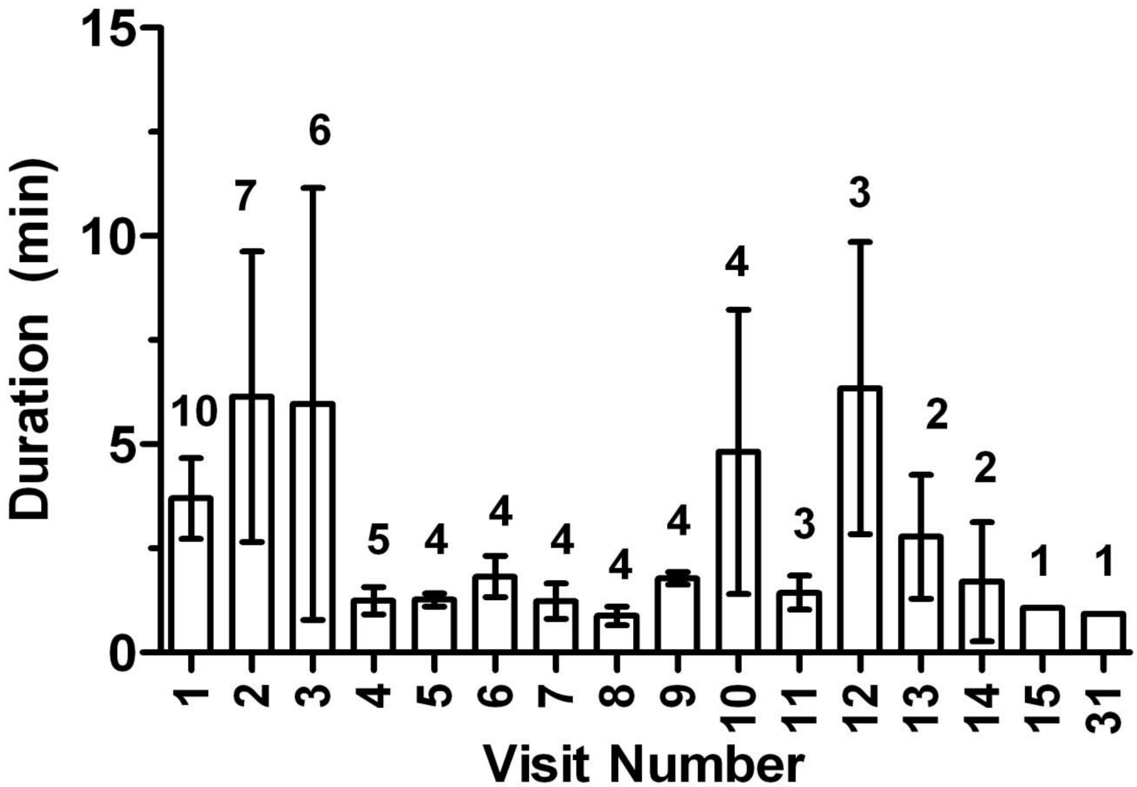

Figure 2. Mean duration ± SE (min) per visit for juvenile Blue catfish, sample size per visit is indicated by number above bar. Note that visit duration for individuals who only entered the bucket once and remained inside were not counted (n=10).

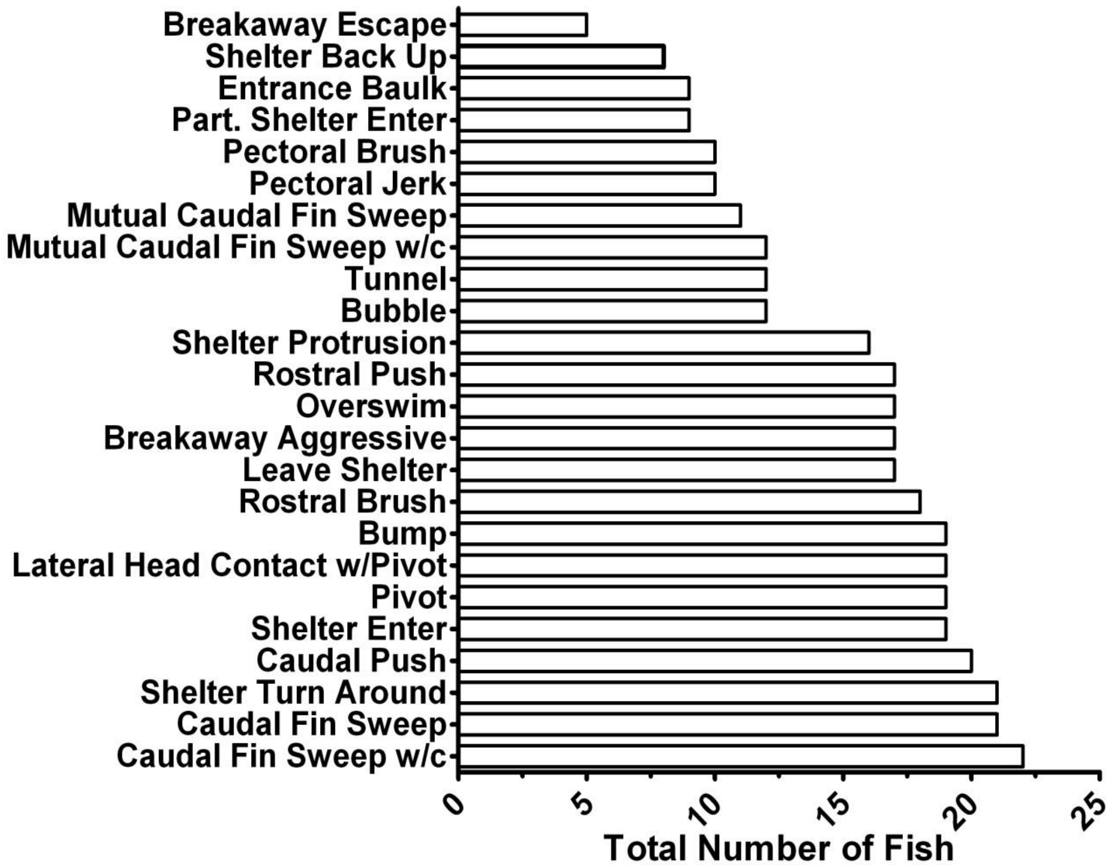

Figure 3. Number of juvenile Blue catfish performing various behaviors in 15 trials. Note that fish are counted twice because of reciprocal pairings. WC=with contact (n=30).

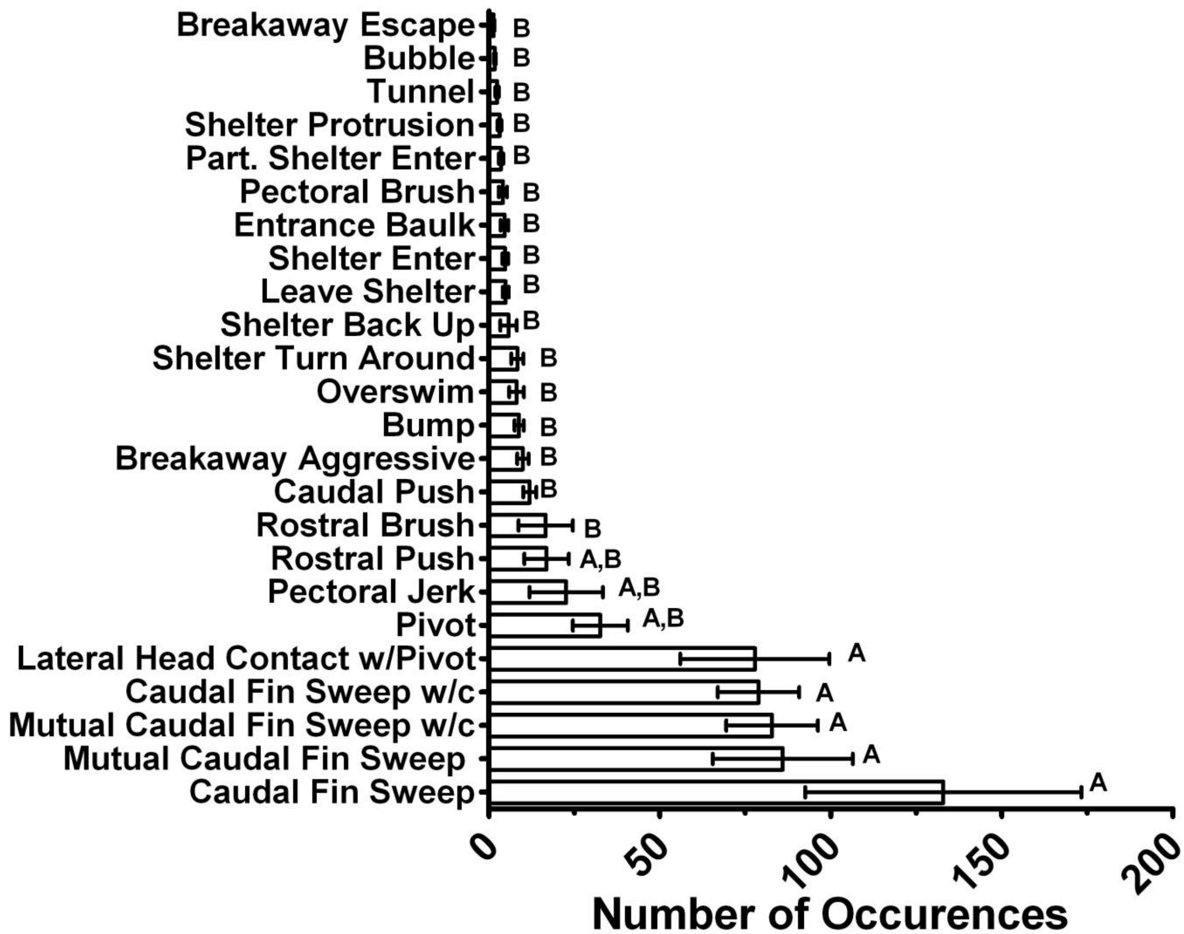

Figure 4. Mean Occurrences ± SE for various behaviors in juvenile Blue catfish. The same letter indicate means that are not significantly different (ANOVA) (n=30).

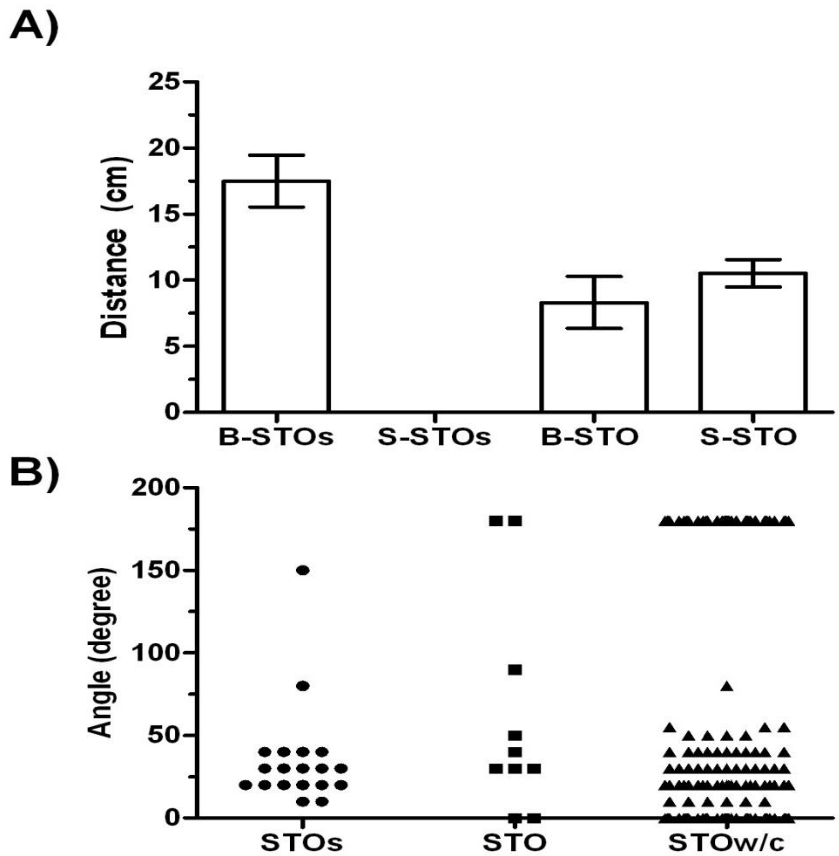

Figure 5. A) Mean distance ± SE (cm) between fish during shelter standoff (STOs) and standoffs (STO) for big (B) and small (S) Blue catfish. **B)** Angle scatter plot standoff type between fish during shelter standoff (STOs), standoff (STO) and standoff with contact (STOw/c). Zero degrees is parallel, 180 degrees is antiparallel. Only fish/trials in which behavior occurred were included (Shelter Standoff n=5, Standoff n=2, Standoff w/c n=6).

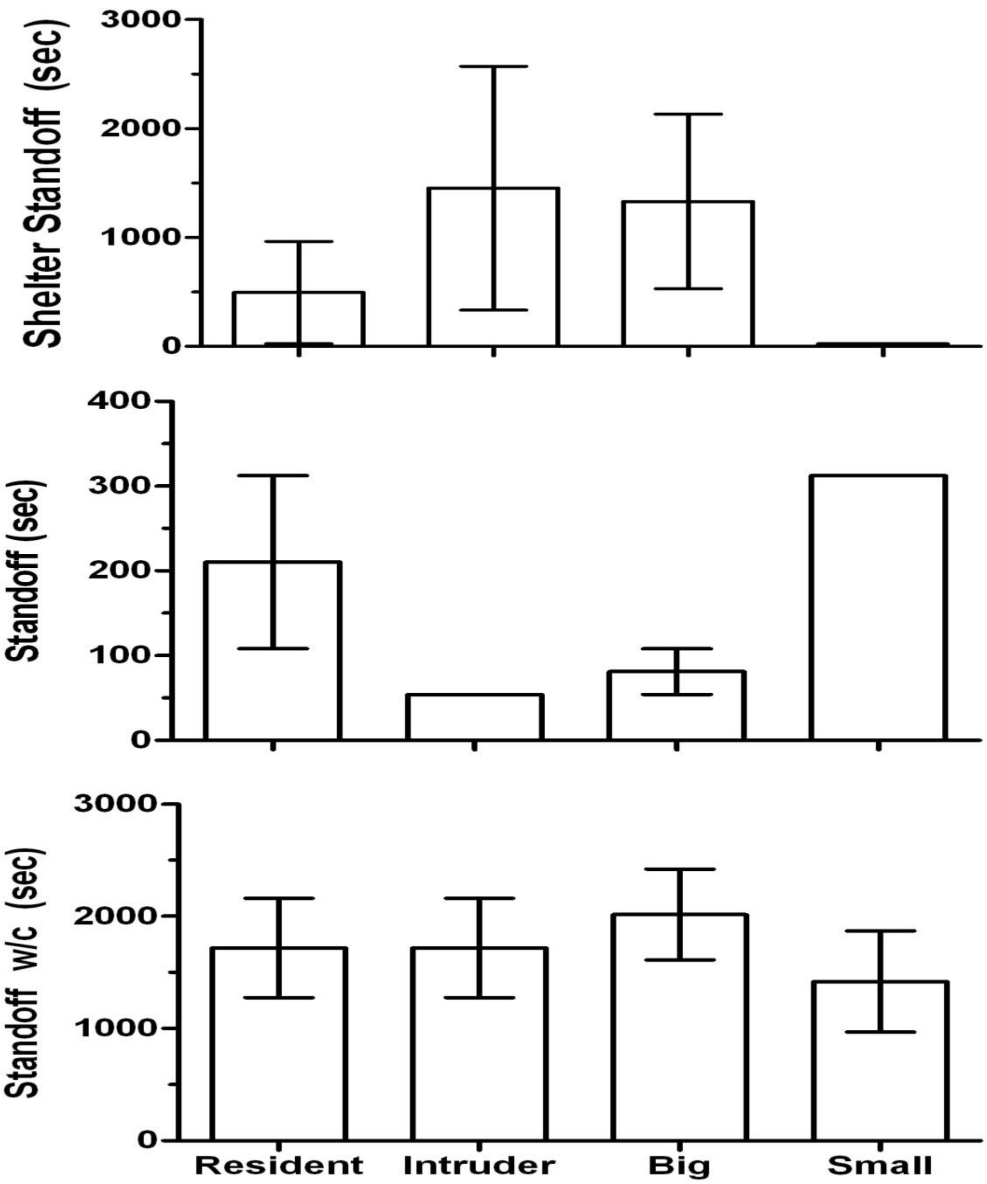

Figure 6. Mean duration ± SE (sec) of standoff behaviors for resident vs. intruder and big vs. small in juvenile Blue catfish. Only fish/trials in which behavior occurred were included (Shelter Standoff n=5, Standoff n=2, Standoff w/c n=6).

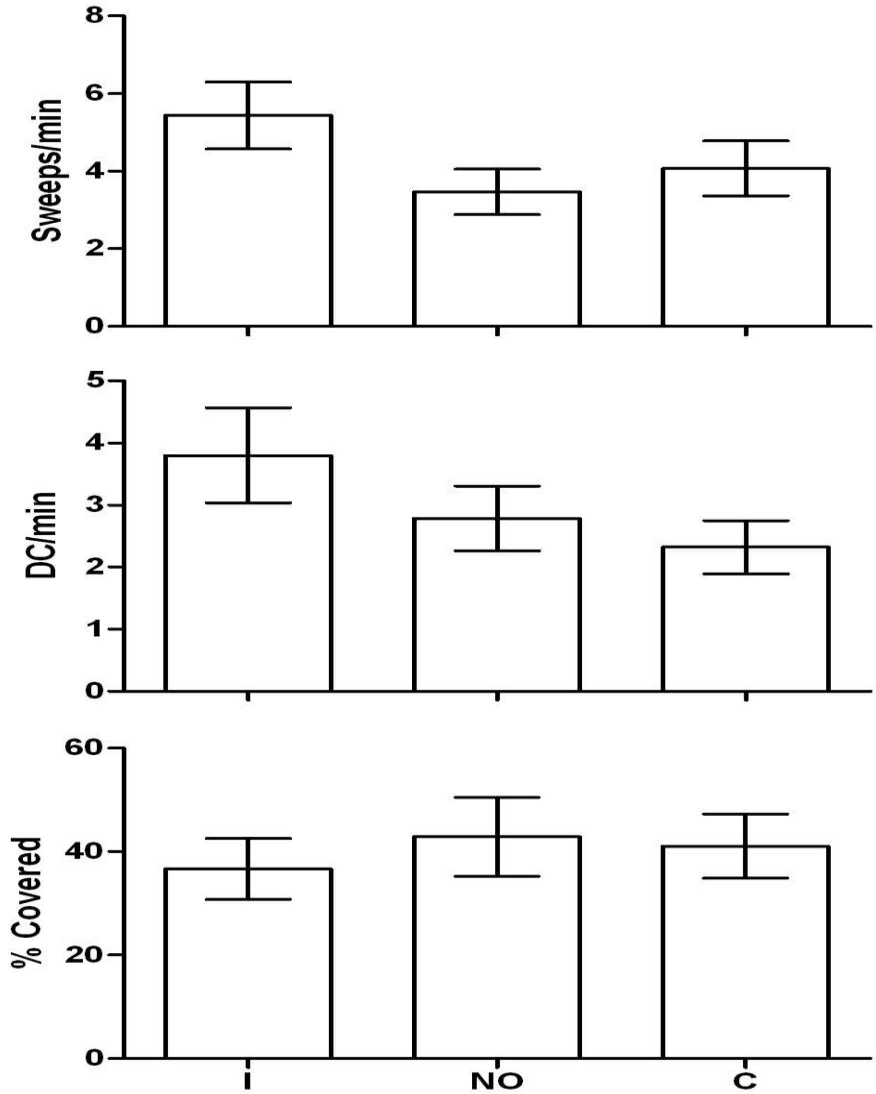

Figure 7. Mean number ± SE of caudal fin sweeps per minute, directional changes of caudal region (DC) per minute, and percent of shelter opening covered with caudal region (% covered) performed during shelter caudal fin sweeps after intruder fish was introduced (I), when it was near the opening (NO), and after contact (C) from intruder in juvenile Blue catfish (n=15).

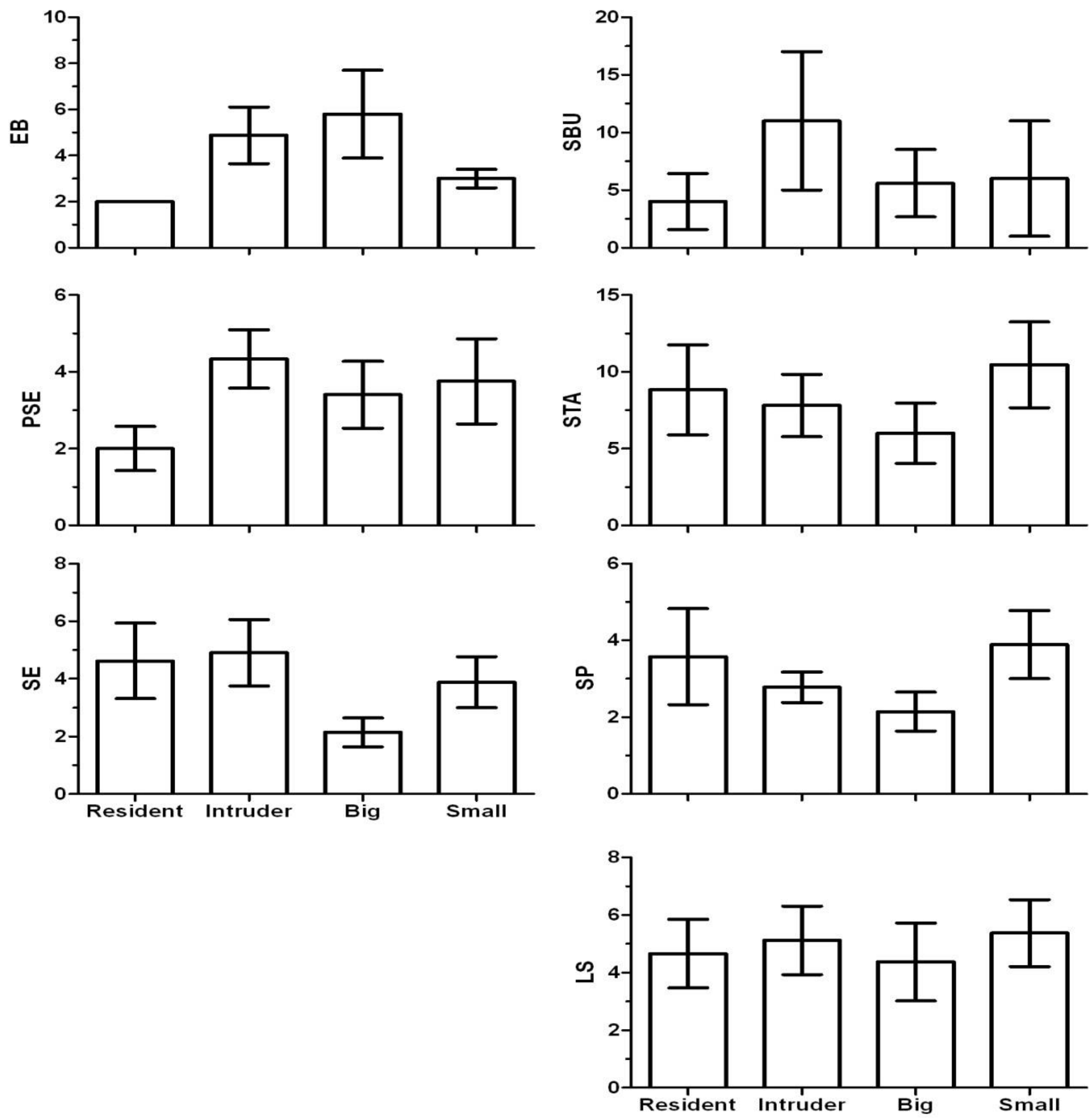

Figure 8. Mean number ± SE of shelter behaviors for resident vs. intruder and large vs. small Blue catfish, including: Entrance Baulk (EB), Partial Shelter Enter (PSE), Shelter Enter (SE), Shelter Backup (SBU), Shelter Turn Around (STA), Shelter Protrusion (SP), and Leave Shelter (LS), (Residents/Intruders n=15, Big n=14, Small n=16).

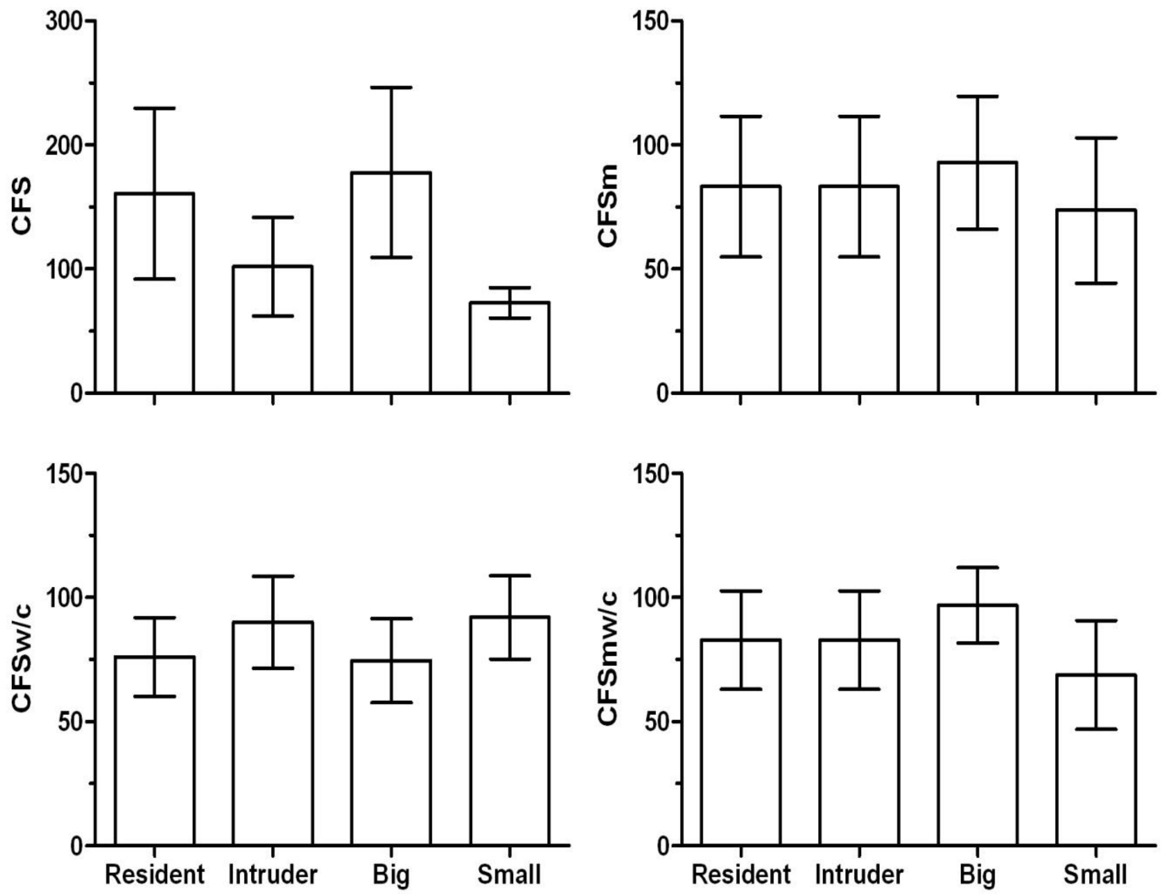

Figure 9. Mean number ± SE of Caudal Fin Sweeps (CFS), Caudal Fin Sweeps with contact (CFSw/c), Mutual Caudal Fin Sweeps (CFSm), an Mutual Caudal Fin Sweeps with contact (CFSmw/c) for resident vs. intruder and large vs. small Blue catfish, including, (Residents/Intruders n=15, Big n=14, Small n=16).

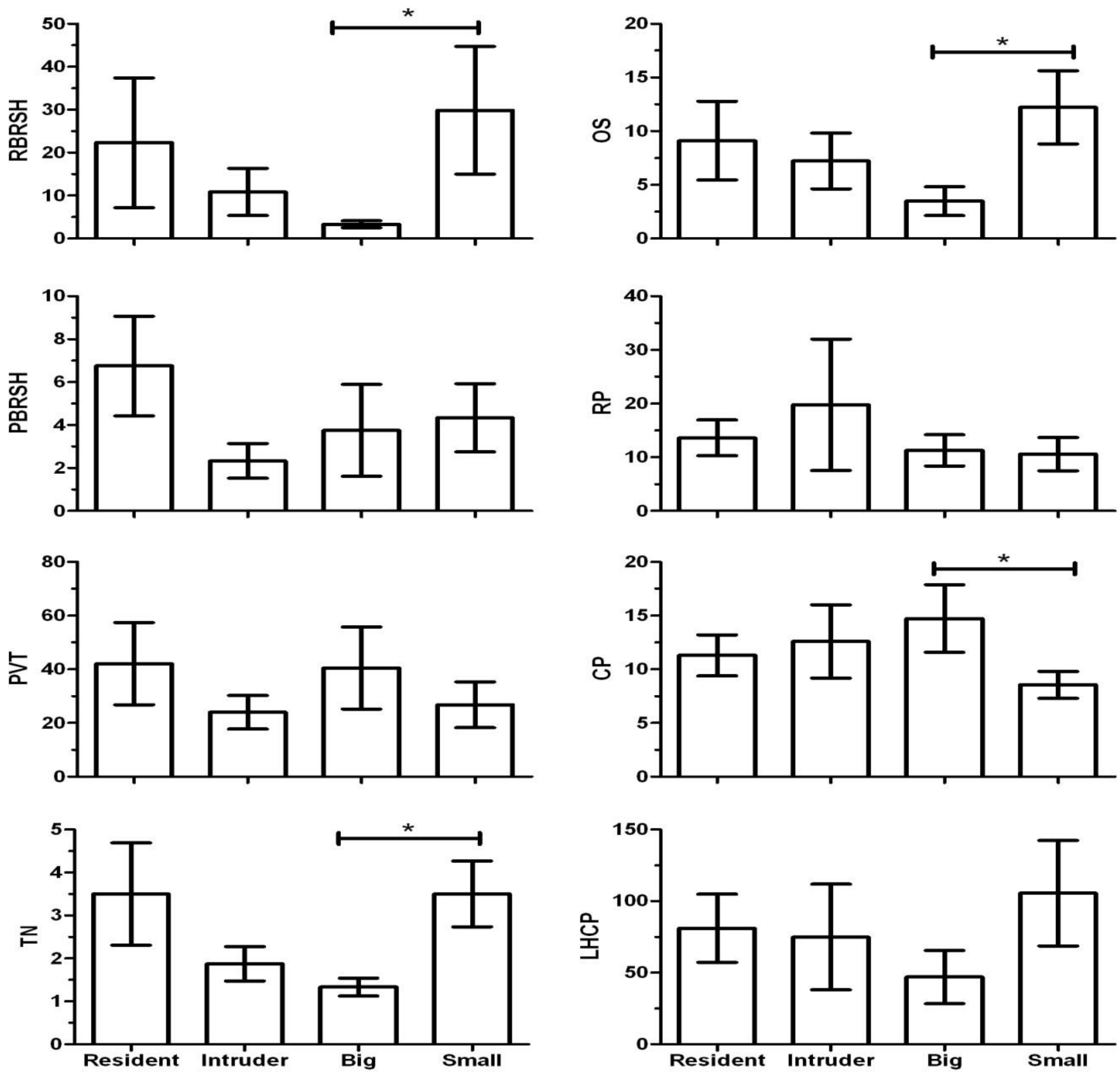

Figure 10. Mean number ± SE of aggressive behaviors for resident vs. intruder and large vs. small Blue catfish, including Rostral Brush (RBRSH), Pectoral Brush (PBRSH), Pivot (PVT), Tunnel (TN), Overswim (OS), Rostral Push (RP), Caudal Push (CP) and Lateral Head Contact with Pivot (LHCP), * indicates significance between 0.01-0.05 (Mann Whitney U test) (Residents/Intruders n=15, Big n=14, Small n=16).

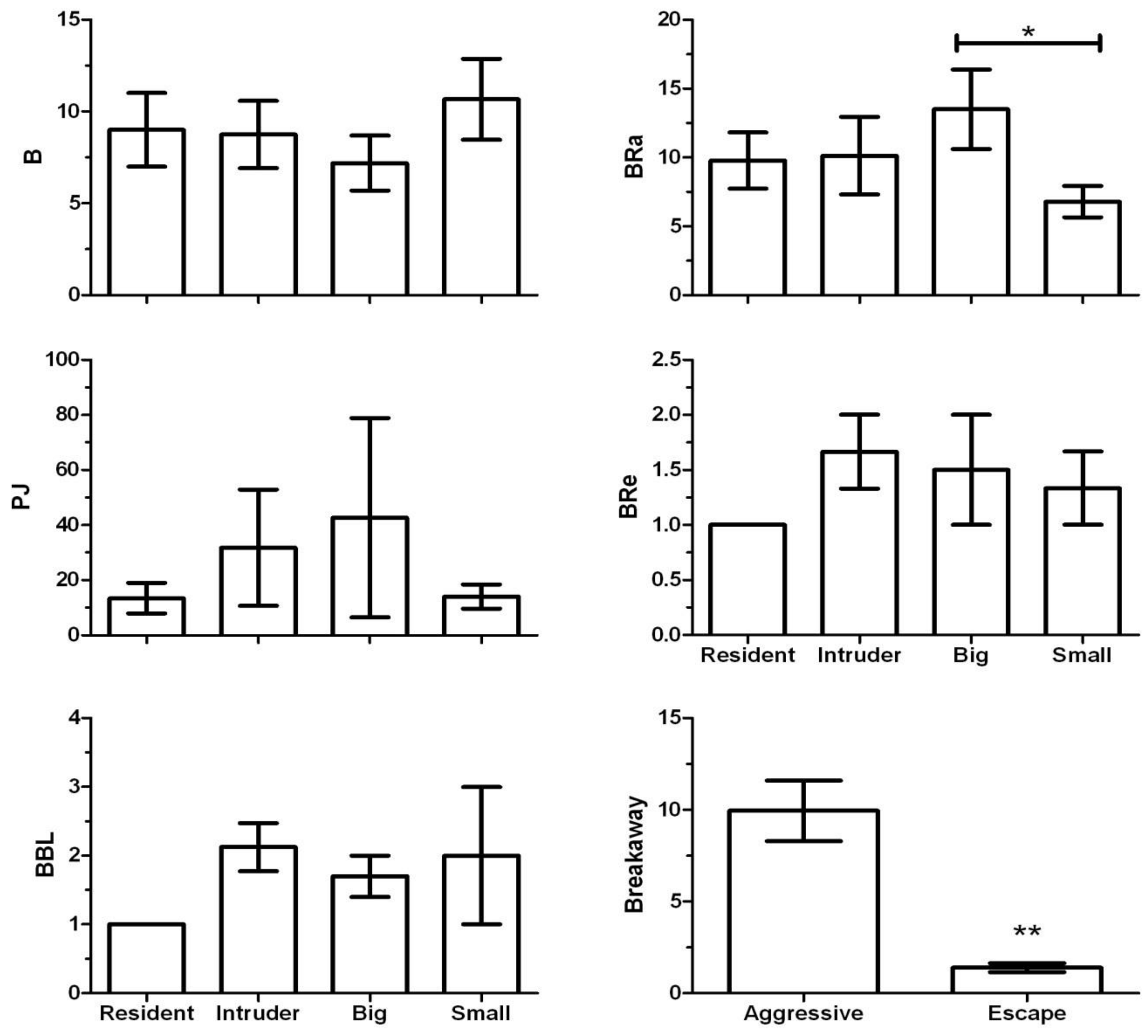

Figure 11. Mean number ± SE of other behaviors for resident vs. intruder and large vs. small Blue catfish, including, Bump (B), Pectoral Jerk (PJ), Bubble (BBL), Breakaway Aggressive (BRa), Breakaway escape (BRe), and a comparison between BRa and BRe, * p<0.05, ** p<0.01 (Mann Whitney U test)(Residents/Intruders n=15, Big n=14, Small n=16).

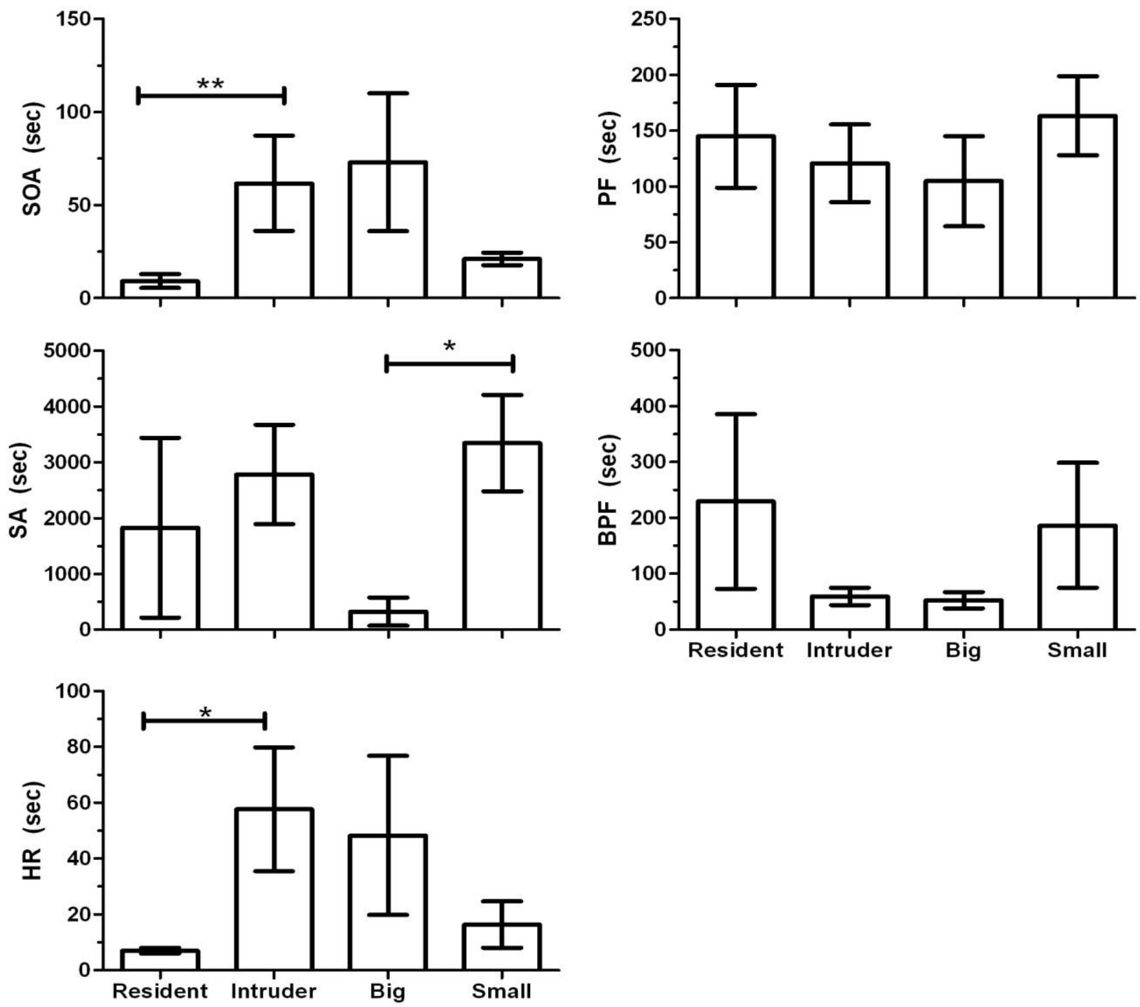

Figure 12. Mean duration ± SE (sec) for resident vs. intruder and large vs. small Blue catfish, including Shelter Orientation and Approach (SOA), Shelter Adjacent (SA), Headrest (HR), Pectoral Fanning (PF), and Backwards Movement with Pectoral Fanning (BPF), * p<0.05, ** p<0.01 (Mann Whitney U test) (Residency n=15, Big n=14, Small n=16).

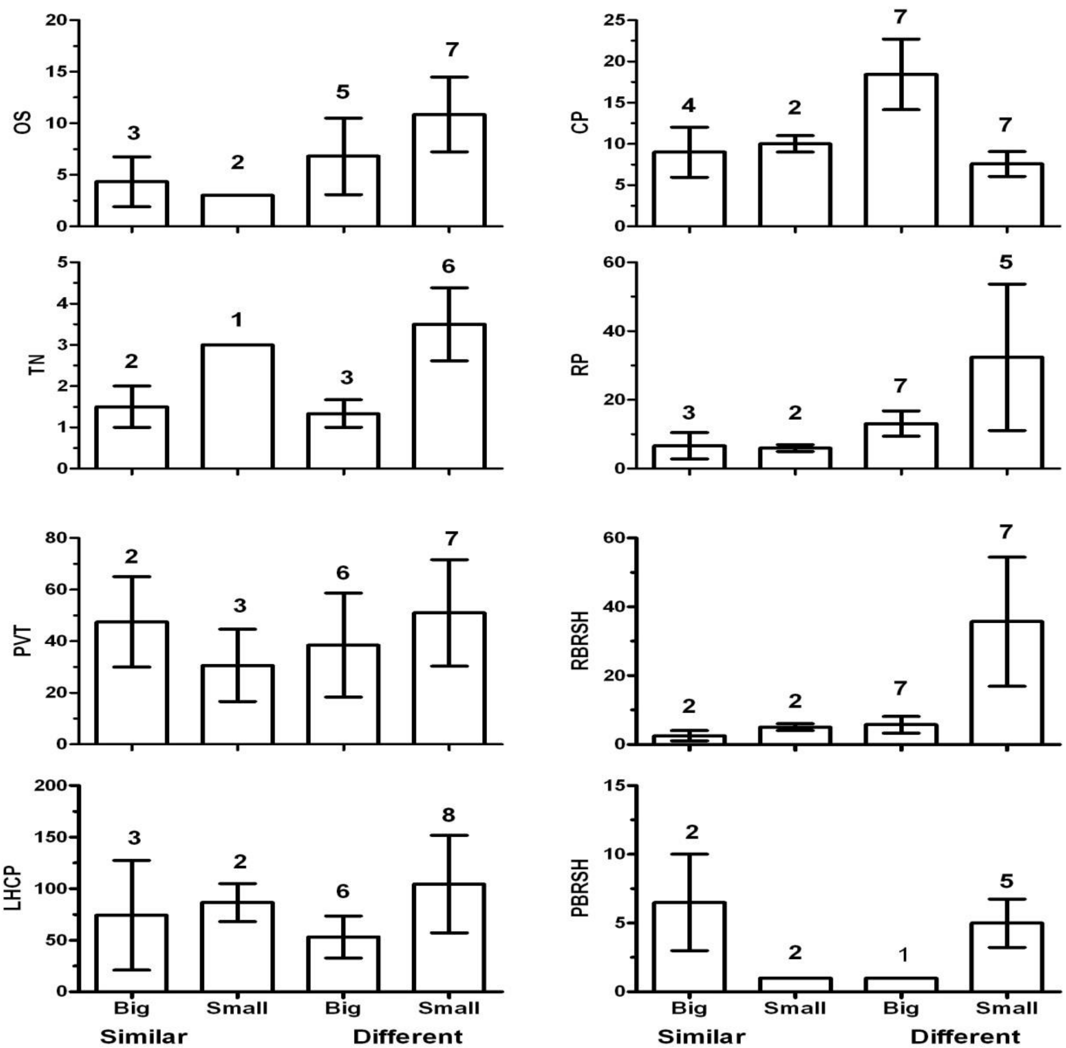

Figure 13. Mean number ± SE Aggressive Behaviors in big and small Blue catfish when paired with similarly sized fish (Big-Big, Small-Small) vs. differently sized fish (Big-Small, Small-Big) including Overswim (OS), Tunnel (TN), Pivot (PVT), Lateral Head Contact with Pivot (LHCP), Caudal Push (CP), Rostral Push (RP), Rostral Brush (RBRSH), and Pectoral Brush (PBRSH) (B-B n=6, S-S/B-S/S-B n=8). Number of fish performing each behavior above bar.

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